STANDARDIZED ILEAL DIGESTIBILITY AND REQUIREMENT ESTIMATES OF AMINO ACIDS IN THREE-WEEK OLD BROILERS

By

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ABSTRACT

Three experiments were conducted to determine the apparent (AID) and standardized (SID) ileal digestibilities of protein and amino acids (AA) in pea protein isolate (PPI), distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) and corn. In addition, two experiments were conducted to estimate the requirements of digestible sulfur amino acids (dSAA) and digestible lysine (dLYS) for 3-week old broilers to obtain optimum growth performance (body weight gain, BWG and feed conversion ratio, FCR) and optimum yield performance (carcass yield, CY, breast meat yield, BMY, and thigh and drumstick yield, TDY). For digestibility studies, day-old male Ross 308 broiler chicks were fed test diets from day 1-14. The test diets were balanced for vitamins and minerals and contained chromic oxide (0.3%) as an indigestible marker. The protein and AA digestibilities were determined either by the direct or indirect method. For requirement studies, day-old male Ross 308 broiler chicks were fed lysine- (Exp. 1) or sulfur AA- (Exp. 2) deficient basal diets balanced for all other AA, except for the AA under test, according to the ideal protein concept. All test diets were also balanced for vitamins and minerals and were kept isonitrogenous and isocaloric within each assay. The average SID estimates of AA were 92.4%, 71.5% and 93.3% in PPI, wcDDGS and corn, respectively. Using regression analysis, the dietary dLYS requirement estimate was 1.12% and 1.13% for optimum BWG and FCR, respectively. The dietary dSAA requirement estimates was 0.81% for optimum BWG, while no significant response was observed for optimum FCR. Both CY and BMY

increased cubically with increasing levels of dLYS, whereas, CY did not respond significantly and BMY increased linearly with increasing levels of dSAA.

DEDICATION

This thesis is dedicated to my mother in law, Joginder Paul Kaur.

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FOREWORD

This thesis was written in Manuscript style and is composed of two Manuscripts, Part of Manuscript I was presented at the Poultry Science Association in North Carolina (2009) and Prairie Poultry meeting in Saskatoon, Saskatchewan (2009). Both manuscripts were written according to the guidelines of Poultry Science manuscript preparation. Authors to all manuscripts are N. Nandha, G. Crow, R. L. Payne, W. Guenter and C. M. Nyachoti.

TABLE OF CONTENTS

Page

ABSTRACT.....i

DEDICATION..... iii

ACKNOWLEDGEMENT.....iv

FOREWORD..... vi

TABLE OF CONTENTS.....vii

LIST OF TABLES.....x

LIST OF ABBREVIATIONS.....xii

1.0 GENERAL INTRODUCTION.....1

2.0 LITERATURE REVIEW.....7

2.1 AA digestibility as an estimator of AA availability.....7

2.2 Digestibility assays.....8

2.2.1 Excreta/ total tract digestibility.....9

2.2.2 Ileal digestibility.....11

2.3 Factors influencing AA digestibility.....13

2.3.1 Method of AA determination.....13

2.3.2 Dietary protein and amino acid levels.....15

2.3.2.1 Ileal Endogenous amino acid losses.....16

2.3.3 Age of birds.....18

2.4 Standardized ileal digestibility.....19

2.5 Ideal chick protein concept.....20

2.6 Different ideal AA profiles.....22

2.7 Variables in the determination of amino acid requirements.....24

2.8 Methods for determining amino acid requirements.....26

2.9 Review of literature available on digestible lysine requirements estimated in 3-week old broilers.....27

2.10 Review of literature available on digestible sulfur amino acids requirements estimated in 3-week old broilers.....30

2.11 Conclusions.....33

3.0 THESIS OBJECTIVES.....35

4.0 MANUSCRIPT I: STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY IN PEA PROTEIN ISOLATES, DISTILLER'S DRIED GRAINS WITH SOLUBLES OBTAINED FROM A BLEND OF WHEAT AND CORN AND CORN FED TO BROILERS.....36

4.1 Abstract.....36

4.2 Introduction.....37

4.3 Objectives.....42

4.4 Materials and Methods.....42

4.4.1 Diets.....42

4.4.2 Birds and conduct of experiment.....43

4.4.3 Chemical analysis.....44

4.4.4 Digestibility calculations.....45

4.4.5 Statistical analysis.....46

4.5 Results and Discussion.....47

4.6 Conclusion.....64

5.0 MANUSCRIPT II: REQUIREMENT ESTIMATES OF DIGESTIBLE LYSINE AND DIGESTIBLE SULFUR AMINO ACIDS FOR THREE-WEEK OLD BROILERS.....65

5.1 Abstract.....65

5.2 Introduction.....66

5.3 Objectives.....68

5.4 Materials and Methods.....69

5.4.1 Diets.....69

5.4.2 Birds and conduct of experiment.....71

5.4.3 Statistical analysis.....74

5.5 Results and Discussion.....75

5.5.1 Experiment 1.....75

5.5.2 Experiment 2.....81

5.6 Conclusion.....84

6.0 GENERAL DISCUSSION.....86

7.0 GENERAL CONCLUSIONS.....92

8.0 REFERENCES.....94

9.0 APPENDIX.....120

LIST OF TABLES

4.0 MANUSCRIPT I

Table 4.1. Crude protein and AA composition (%) in PPI (n = 4), wcDDGS (n = 5) and corn (n = 5) samples fed to broilers (DM basis).....48

Table 4.2. Apparent ileal protein and AA digestibility coefficients in PPI (n = 4), wcDDGS (n = 5) and corn (n = 5) samples fed to broilers.....51

Table 4.3. Standardized ileal protein and AA digestibility coefficients in PPI (n = 4), wcDDGS (n = 5) and corn (n = 5) samples fed to broilers.....52

Table 4.4. Coefficients of variation (CV%) associated with AID of CP and AA determined in broiler chicks fed diets containing PPI (n = 4), wcDDGS (n = 5) and corn (n = 5) samples.....59

Table 4.5. Coefficients of variation (CV%) associated with SID of CP and AA determined in broiler chicks fed diets containing PPI (n = 4), wcDDGS (n = 5) and corn (n = 5) samples.....60

Table 4.6. Apparent ileal CP and AA digestibility coefficients and their associated CV (%) determined in broiler chicks fed diets containing a reference wheat sample in three different assays.....62

Table 4.7. Standardized ileal CP and AA digestibility coefficients and their associated CV (%) determined in broiler chicks fed diets containing a reference wheat sample in three different assays.....63

5.0 MANUSCRIPT II

Table 5.1. Standardized ileal AA digestibility coefficients, analyzed total AA and calculated digestible AA in wheat and dry-extruded expelled soybean meal (DESBM).....70

Table 5.2. Ingredient composition of lysine and sulfur AA deficient basal diets (as fed basis).....72

Table 5.3. Calculated composition of digestible lysine (Exp. 1) and digestible SAA (Exp. 2) deficient diets..... 73

Table 5.4. Calculated and analyzed total lysine and total SAA contents in experimental diets.....76

Table 5.5 Growth and yield performance of broilers fed graded levels of digestible lysine from 0 to 21 days of age.....77

Table 5.6. Estimated requirements (based on quadratic regression analysis) of digestible lysine and SAA in 3-week old broilers.....78

Table 5.7. Growth and yield performance of broilers fed graded levels of digestible sulfur AA from 0 to 21 days of age.....82

9.0 APPENDIX

Table 9.1. Basal ileal endogenous AA flow (mg/kg DM intake) in chicks fed a nitrogen-free diet.....120

Table 9.2. Estimated requirements (based on quadratic regression analysis) of digestible lysine and digestible SAA in 3-week old broilers.....121

LIST OF ABBREVIATIONS

AA	Amino acid(s)
AID	Apparent ileal digestibility
Ala	Alanine
ANF	Anti-nutritional factor(s)
Arg	Arginine
Asp	Aspartic acid
BMY	Breast meat yield
BWG	Body weight gain
CP	Crude protein
CV	Coefficient of variation
CY	Carcass yield
Cys	Cysteine
DDGS	Distillers'dried grains with solubles
DESBM	Dry-extruded expelled soybean meal
dLYS	Digestible lysine
DM	Dry matter
dSAA	Digestible sulfur amino acid (s)
Exp	Experiment
FCR	Feed conversion ratio
Glu	Glutamic acid

Gly	Glycine
His	Histidine
HDP	High density protein
Ile	Isoleucine
IEAL	Ileal endogenous amino acid losses
IICP	Illinois ideal chick protein
ICP	Ideal chick protein
Leu	Leucine
Lys	Lysine
Met	Methionine
NFP	Nitrogen free diet
NSP	Non-starch polysaccharides
Phe	Phenylalanine
PPI	Pea protein isolate(s)
Pro	Proline
SAA	Sulfur amino acid(s)
Ser	Serine
SID	Standardized ileal digestibility
Thr	Threonine
TID	True ileal digestibility
TDY	Thigh and drumstick yield
Val	Valine

1.0 GENERAL INTRODUCTION

Proteins are the most vital nutrients of food, acting both as a source of energy as well as the main component for growth, production and maintenance. Therefore, it is of utmost importance to assess the dietary proteins, or more clearly the AA profile of feed ingredients, in order to evaluate their nutritional value (Stein et al., 2007a). The AA availability rather than total AA content is considered as a more accurate measure to determine the nutritive value of a feed ingredient (Rostagno et al., 1995). Researchers have observed that birds fed diets formulated based on digestible AA content performed better than the birds fed diets formulated based on total AA content (Green et al., 1987; Fernandez et al., 1995; Rostagno et al., 1995; Wang and Parsons, 1998; Douglas and Parsons, 1999; Farrell et al., 1999). Because there is no direct and satisfactory method to estimate the AA availability (Stein et al., 2007a), the digestibility is regarded as a sensitive and reliable estimate of AA availability (Sauer and Ozimek, 1986; Laplace et al., 1989; Ten Doeschate et al., 1993; Rostagno et al., 1995; Moughan, 2003).

As the dietary proteins enter the small intestine they get mixed with endogenous proteins (from enzymes, mucoproteins, desquamated epithelial cells, serum albumin, peptides, AA, amides, amines) and both the dietary and endogenous proteins together are subjected to digestive and absorptive processes in the small intestine (Nyachoti et al., 1997; Mosenthin et al., 2000; Moughan, 2003). Digested free AA and small peptides get

absorbed while undigested and unabsorbed protein products are left. About 70 to 80% of endogenous nitrogen is reabsorbed while 20 to 30% of endogenous proteins (mainly mucin) along with other undigested and unabsorbed dietary proteins enter the large intestine and undergo fermentation by the microflora present in the hindgut, mainly the caecum (Mosenthin et al., 2000; Fuller, 2003). The cecal microbes either deaminate nitrogenous substances (dietary and non-dietary origin) (Salter and Fulford, 1974) or synthesize microbial proteins (Payne et al., 1968; Parsons, 1986). Deamination of AA leads to the formation of ammonia, which is absorbed from the large intestine, but is not available for utilization by the bird since it is lost as uric acid (Salter and fulford, 1974). In this case, the fecal analysis method overestimates the AA digestibilities. On the other hand, due to microbial net protein synthesis, the fecal analysis method underestimates the AA digestibilities (Mosenthin et al., 2000). Consequently, microbial action on proteins in the large intestine interferes with accurate measurement of protein and AA digestibilities. Most of the AA digestibility values published in the literature has been determined with adult cockerels/roosters using the Sibbald precision feeding assay (Sibbald, 1979) or using its modified version (Parsons, 1985). The precision feeding assay has obvious advantages of simplicity (birds need not to be sacrificed), and ease of using the same birds repeatedly. However, the differences observed in digestive capabilities of birds with age (Noy and Sklan, 1995; Batal and Parsons, 2002) as well as differences in AA digestibility values due to methodology (Fan and Sauer, 1995; Garcia et al., 2007) questions the application of digestibility values from adult roosters to young broilers. Moreover, the excreta AA analysis done in a precision feeding assay does not represent

the true picture of AA digestibility as stated earlier. Therefore, it is more accurate to measure the AA digestibilities in ileal digesta than in excreta (Payne et al., 1968; Ravindran et al., 1999; Kadim et al., 2002).

If the ileal analysis method is to be used as a precise tool of determining dietary AA digestibilities, then endogenous AA losses should be determined since ileal digesta contains endogenous proteins (Mosenthin et al., 2000). Ileal endogenous AA losses (IEAL) are of two types, non-specific losses (basal or diet-independent) and specific losses (diet dependent) (Nyachoti et al., 1997; Ravindran and Bryden, 1999; Lemme et al., 2004). The basal IEAL are related to dry matter intake, whereas, the specific endogenous losses are related to the composition of a feedstuff (Lemme et al., 2004). When the AA digestibility coefficients are determined from ileal digesta without taking into account the endogenous AA fractions, they are called apparent ileal AA digestibility coefficients. The correction of AID coefficients for the basal endogenous AA losses and specific endogenous AA losses are categorized as standardized and true AA digestibility coefficients, respectively. The IEAL are believed to vary with age and method of determination (Ravindran and Hendriks, 2004; Adedokun et al., 2007). Consequently, the AID values of AA vary with age (Ten Doeschate et al., 1993; Batal and Parsons, 2002; Huang et al., 2005; Adedokun et al., 2007; Garcia et al., 2007) and method of AA determination (Fan and Sauer, 1995; Ravindran and Bryden, 1999; Mosenthin et al., 2000). Also, the genotype (Huang et al., 2006; Adedokun et al., 2009) and sex of the bird used for the assay (Ten Doeschate et al., 1993) influences the AA digestibility values. Hence, it is imperative to correct the AID values to TID or SID values. Actually, the true

ileal digestibility (TID) is a fundamental property of a feed ingredient since it considers both specific and non-specific losses and is independent of experimental and dietary conditions. However, procedures for determining total IEAL (both specific and non-specific) cannot be performed routinely. In contrast, standardized values are independent of assay conditions, and are additive in a mixed diet (Angkanaporn et al., 1996; Lemme et al., 2004; Stein et al., 2001, 2005); therefore, standardized ileal digestibility measurements have been regarded as an appropriate approach to describe AA digestibility for broiler feed formulation (Stein et al., 2007b).

Although digestible AA values are available their use in poultry feed formulation is still uncommon. This is not only because of the wide variations (due to type of birds, feed samples, assay diets and assay methodology) among the published digestible AA values, but also because of insufficient knowledge of batch to batch variation of AA digestibility values (Ravindran and Bryden, 1999; Mosenthin et al., 2000; Lemme et al., 2004). The variations observed in AID of proteins and the AA between and within samples of the same feedstuff could be explained in terms of feed intake levels by birds or methodologies used. Therefore, along with addressing the digestibility data, the CV among the different samples of a feed ingredient due to external (assay conditions) or internal (feed composition) factors was also studied to account for the source of variations. This information on digestibility values and their associated CV would improve precision in formulating diets which are cost effective, have narrow margins of safety and decrease nitrogen excretion into the environment.

Therefore, the first objective of this research study was to determine the AID and SID of proteins and AA along with the associated CV among samples of three feed ingredients (pea protein isolates (PPI), distiller's dried grains with soluble derived from a blend of wheat and corn (wcDDGS) and corn) when fed to 21-day old broilers.

The AA requirements of broilers have been reported to vary due to environmental, dietary and genetic factors; therefore, it is not possible to make an accurate estimate of requirements for all AA under all combinations of conditions and genotypes. However, it has also been assumed that dietary requirements for each of the indispensable AA can be expressed in fixed proportion to the requirement for the other indispensable AA (Han and Baker, 1994; Baker, 1997; Emmert and Baker, 1997; Mack et al., 1999; Baker et al., 2002). Considering these facts, nutritionists proposed the concept of "ideal AA ratios" which refers to the blend of indispensable AA in a diet known to satisfy the need without any deficiencies or excesses (Emmert and Baker, 1997). The advantage of applying this concept in meeting the AA requirements of an animal is that once the ideal ratios are established for a particular age or period, further research determining AA requirements under a variety of conditions need to concentrate only on the requirement estimate of the reference AA, and the requirements for the remaining AA can be calculated relative to the reference AA. This reduces extra AA input (more than required), minimizes feed cost and nitrogen excretion into the environment (Baker, 1997; Emmert and Baker, 1997). Also, the ideal protein opens windows for incorporation of alternative feed ingredients in practical broiler diets without requiring the determination of total AA requirements for these diets (Emmert and Baker, 1997). It is recommended

that ideal AA ratios should be established using digestibility coefficients to overcome the differences in dietary AA absorption and utilization, which can cause variations in AA proportions (Baker, 1997). Several studies have demonstrated the beneficial effects of formulating broiler diets using digestible values and ideal protein ratios when poorly digestible ingredients are incorporated.

Therefore, the second objective of this research was to estimate the requirements of dLYS and dSAA for optimum growth and yield performance in 21-day old broilers when all the indispensable AA, except AA under test were in proportion according to the ideal protein concept.

2.0 LITERATURE REVIEW

2.1 AA DIGESTIBILITY AS AN ESTIMATOR OF AA AVAILABILITY

For more accurate feed formulation, the protein value of a feed ingredient should be expressed as available AA rather than the total AA (Stein et al., 2007a). Several methods have been developed to evaluate the availabilities of protein and AA in feed ingredients for poultry. These methods are *in vitro* (enzymic, chemical or microbiological assays), indirect *in vivo* (plasma AA assays), or direct *in vivo* (growth or digestibility assays) (Sibbald, 1987; Ravindran and Bryden, 1999). Among these methods, direct *in vivo* were considered most precise. The growth assay (also known as slope-ratio technique) (Batterham et al., 1979) was found to be time consuming (only one AA can be assessed at a time), expensive for routine measurement, and has a high standard error of the mean (Batterham, 1992) when used to measure AA availability in pigs. Therefore, the digestibility assay gained popularity as a suitable and sensitive measure of availability (Laplace et al., 1989; Ravindran et al., 1999; Lemme et al., 2004; Garcia et al., 2007; Stein et al., 2007a). The terms availability and digestibility are often used synonymously, but they have been defined differently (Batterham, 1992). Availability measurements accounts not only for digestion and absorption, but also, provide information about utilization of a nutrient. On the other hand, digestibility measurements define only the digestion and the absorption of a nutrient, with no clue about the fate of a nutrient at the tissue level. According to Batterham (1992) the AA bioavailability is the degree to which

AA has under gone the processes of digestion, absorption and metabolism, or in other words, the proportion of AA in diet that are absorbed in a form suitable for utilization by the animal. And the digestibility is the process of enzymatic hydrolysis and microbial fermentation of ingested proteins and peptides followed by absorption of AA and peptides from the gastrointestinal lumen (Fuller, 2003). Therefore, AA digestibility values might not be reliable representatives of AA availability values if feed ingredients assayed are of poor quality. For instance, it has been reported that AA digestibility and AA availability estimates are similar in case of high quality feedstuffs while these estimates differ in case of low quality ingredients Achinewhu and Hewitt, 1979; Batterham, 1992). Also, not all the absorbed AA are available for utilization, for instance, early maillard reaction products of AA such as lysine and cystine (Parsons, 1992). Besides these constraints, digestibility assays are the most common and favoured technique for measuring AA availability, because these assays are inexpensive, easy to use on routine basis, measures all AA at once, and applies directly to an animal (Johnson, 1992; Ravindran and Bryden, 1999).

2.2 DIGESTIBILITY ASSAYS

Digestibility assays are divided into excreta/total tract (using intact or cecectomised birds) and ileal digestibility (slaughtered or ileal cannulated birds) assay.

2.2.1 EXCRETA/TOTAL TRACT DIGESTIBILITY

The excreta collection method involves, either the total collection of excreta for an appropriate period of time after feeding, or, random collection of the excreta sample after the use of markers in the diet (Ravindran and Bryden, 1999). The collected excreta samples are analyzed for all AA and differences between the amounts of each AA consumed and excreted are measured to estimate the AA digestibilities (Sauer and Ozimek, 1986). Most of the published data on excreta AA digestible values of feed ingredients in poultry was created using the precision feeding cockerel assay of Sibbald (1979) or a modification of this assay (Parsons, 1981, 1985). According to Sibbald's assay, a precise amount of test feedstuff is intubated in the crop of adult cockerels fasted previously for 24 to 48 hrs. The total excreta from these birds are then collected to determine AA digestibilities. The advantages of an excreta analysis method are its simplicity, the efficiency to test many raw materials in a short time, the ease to carry on large number of birds and the reuse of cockerels (McNab, 1994). Despite the advantages, the use of excreta as the means of determining digestibility coefficients has been criticized due to its many drawbacks (Payne et al., 1968; Salter and Fulford, 1974; Parsons et al., 1981, 1983b, 1986; Raharjo and Farrell, 1984). The first drawback is that, excreta-based measurements do not take into account the effects of hindgut micro-organisms on protein digestion/ utilization and thus the resultant contribution of microbial proteins to the AA profile of excreta (Parsons, 1986; Ten Doeschate et al., 1993). Second, fasting and force-feeding are not the normal physiological processes (Ravindran and Bryden, 1999). Third, uric acid contamination may affect excreta

digestibility (Ten Doeschate et al., 1993; Ravindran et al., 1999). However, it has also been reported that contribution of urinary AA in poultry excreta is negligible (McNab, 1994). Fourth, the applicability of the data generated using adult cockerels to young growing chicks is questionable since AA digestibilities vary with age (Ten Doeschate et al., 1993; Noy and Sklan, 1995; Batal and Parsons, 2002; Huang et al., 2005; Garcia et al., 2007).

The net result of microbial metabolic activity on the AA digestibility depends upon the amount and type of dietary AA (Salter and Fulford, 1974; Angkanaporn et al., 1997) and dietary carbohydrate contents entering the cecum (Parsons, 1983a; Sauer and Ozimek, 1986). The published reports (Sauer et al, 1977; Parsons et al., 1982, 1984; Green et al., 1987; Angkanaporn et al., 1997; Ravindran et al., 1999; Nouri- Emamzadeh et al., 2009) on excreta AA digestibility have concluded that, if undigested AA are deaminated /degraded by hindgut microbes, the recovery of AA in excreta will be lower, resulting in the overestimation of AA digestibilities. On the other hand, if undigested AA are used as substrates for microbial protein synthesis, the recovery of AA in excreta will be higher, resulting in underestimation of digestibilties. Therefore measurement of excreta digestibility is not a precise estimator of AA digestibility.

The cecum is the main site of bacterial activity in the hindgut which significantly influences AA excretion in poultry; therefore, cecectomy was suggested as a tool to overcome the modifying action of cecal microbes on dietary proteins in the large intestine. Published reports show variable results on use of cecectomy. While some

authors favoured this technique (Parsons, 1984; 1986; Johns et al., 1986; Green et al., 1987; Angkanaporn et al., 1997; Nouri-Emamzadeh et al., 2009; Nouri-Emamzadeh and Yaghobfar, 2009) others doubted it (Whitacre and Tanner, 1989; McNab, 1994). Moreover, as reported by Sakata (1987) if short chain fatty acids which are end products of cecal microbial fermentation, are regulating the digestive processes in gastrointestinal tract, the practice of cecectomy is not favourable.

2.2.2 ILEAL DIGESTIBILITY

Researchers have compared the ileal and excreta method of AA digestibility (Ravindran et al., 1999; Kadim et al., 2002) and have regarded the ileal digestibility assay as a more reliable predictor of dietary protein quality and AA digestibility as well as a precise estimator of broiler performance (Payne et al., 1968; Kadim and Moughan, 1997a, b; Ravindran et al., 1999; Kadim et al., 2002).

Since, most of the dietary AA are absorbed either in or before the ileum (Webb, 1990), the terminal ileum is a perfect site for the digesta collection (Raharjo and Farrell, 1984; Laplace et al., 1989). Ileal digesta can be obtained either by slaughtering the birds and manually collecting digesta from the distal ileum or by inserting a T-cannula in the distal ileum of adult cockerels (Raharjo and Farrell, 1984; Ravindran and Bryden, 1999). In the slaughter method, birds are killed humanely, and the small intestine is exposed to separate the ileum which corresponds to the part of the small intestine extending from the Meckel’s diverticulum to 2 cm proximal to the ileo-cecal junction (Ravindran et al.,

1999; Lemme et al., 2004). Kadim and Moughan (1997a) stated that the suitable site for sampling ileal digesta from the broiler chicken is the terminal 15 cm of the ileum. The digesta from several birds within a cage is pooled by gentle flushing using the distilled water to obtain sufficient sample for analysis. In the ileal cannulation method, adult cockerels are surgically opened and a T- piece cannula is placed in the distal ileum (Raharjo and Farrell, 1984; Gurnsey et al., 1985). Although, this method is practical and allows the reuse of experimental birds, yet it has many drawbacks such as the rejection of cannula, the need to use appropriate markers, and the physical alteration to the intestine which may interfere with normal physiological processes of the animal (Tanksley et al., 1981). Thus, the slaughter method of ileal digesta collection is preferred over the ileal cannulation. To eliminate the need of collecting total digesta, an indigestible marker (e.g. chromic oxide, titanium oxide, acid-insoluble ash) which does not affect the nutrient digestibility and which can be completely recovered, is added to the test diets. Chromic oxide has been shown to be a suitable marker (Achinewhu and Hewitt, 1979; Raharjo and Farrell, 1984; Johns et al., 1986; Ten Doeschate et al., 1993). The AA digestibility coefficient (DC) is calculated using the following formula

[1] $DC\ (\%) = 100 - ((M_{diet} \times AA_{digesta}) / (M_{digesta} \times AA_{diet}) \times 100)$,

where M_{diet} and $M_{digesta}$ are the concentrations of marker (mg/kg DM) in the diet and digesta, respectively, and AA_{diet} and $AA_{digesta}$ are the concentrations of AA (mg/kg DM) in the diet and in the digesta respectively.

2.3. FACTORS INFLUENCING ILEAL AA DIGESTIBILITY

2.3.1 Method of AA digestibility determination

The AA digestibility in a feedstuff can be determined using the direct, difference or regression methods. In the direct method, the assay diet is balanced for vitamins and minerals and the requirements of energy are met using oils, dextrose or starch. The test feed ingredient is the sole source of dietary protein in the assay diet (Lin et al., 1987; Fan and Sauer, 1995). Therefore, the AA digestibility coefficient in the test feed ingredient is actually the AA digestibility value in the assay diet which is calculated according to the equation [1] stated previously.

The difference method involves the formulation of a basal and an assay diet. The basal diet contains a basal feed ingredient, which provides the sole source of dietary AA. The assay diet consists of a mixture of the basal diet and a test feed ingredient (Van Leeuwen et al., 1987). Assuming that there are no interactions between the digestibility values of AA in the basal and test feed ingredient, the relationship between digestibility values can be expressed according to the equation [2]. The digestibility value of an AA in the test feed ingredient can then be determined according to the equation [3] (Van Leeuwen et al., 1987)

[2] $D_D = (D_B \times S_B) + (D_A \times S_A)$

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

D_D = AID of an AA in the assay diet (%); D_B = AID of an AA in the basal feed ingredient (%); S_B = contribution level of an AA from the basal feed ingredient to the assay diet, and $S_B = 1- S_A$ (%); D_A = AID of an AA in the assay feed ingredient (%); and S_A = contribution level of an AA from the assay feed ingredient to the assay diet (%).

The regression method evaluates simultaneously the digestibility values of AA in the basal and the assay feed ingredient. Similarly to the difference method, it is assumed that there are no interactions between the digestibility values of AA in the basal and the assay feed ingredients. The basal and the assay feed ingredients are mixed at graded levels to formulate a series of assay diets. The linear relationships between the ileal digestibility value of an AA in the assay diet and the contribution levels of AA from the basal and assay feed ingredient to the assay diet can be expressed according to the equation [4] (Fan and Sauer, 1995).

[4] $D_{Di} = D_A + (D_B – D_A) \times S_{Bi}$

D_{Di}, D_B and D_A = AID of an AA in the ith assay diet (%), basal feed ingredient (%) and a test feed ingredient (%), respectively; S_{Bi} = contribution level of an AA from the basal feedstuff to the ith assay diet, $S_{Bi} = 1- S_{Ai}$; and S_{Ai} = contribution level of an AA from the test feedstuff to the ith assay diet.

The method of determination is an important factor responsible for variation in ileal protein and AA digestibility values reported for samples of the same feed ingredient. Fan and Sauer (1995) evaluated three methods for the determination of AID values of AA in barley and canola meal for swine. They observed that AID of AA in barley when

determined with the difference method were significantly lower than the AID of AA determined by the direct method, while no differences for AID of AA in canola meal were noted by either method. Therefore it was suggested that in order to eliminate these variations, the method of determination specifically suited for a feed ingredient should be used to determine AA digestibility. For example, if it is a high-protein feed ingredient any of the direct, difference and regression methods can be used, if it is a low-protein feed ingredient either the difference or the regression methods are appropriate and if it is a poorly palatable feed ingredient, the regression method should be used to measure digestibility (Fan and Sauer, 1995; Mosenthin et al. 2000).

2.3.2 Dietary protein and AA levels

The differences in protein and AA levels in samples of the same feed ingredient or between different feed ingredients can also lead to variations in ileal AA digestibility values (Sibbald, 1979; Green et al., 1987; Fan and Sauer, 1999; Short et al., 1999). The feed intake levels (Kadim and Moughan, 1997b) and more precisely protein intake levels (Fan et al., 1994; Angkanaporn et al., 1997) have been shown to influence the AID values. The AID of AA increases with increase in dietary protein level till a plateau is obtained after which AID coefficients become independent of dietary AA levels (Fan et al., 1994; Fan and Sauer, 1999; Lemme et al., 2004). Therefore it can be said that the AID values are most affected in low protein feed ingredients such as cereals and grain legumes and are least affected in high protein ingredients (Fan et al., 1994). This is because the low intake of dietary protein causes release of endogenous AA which get

mixed with dietary AA and thus contribute to total digesta (Kadim and Moughan et al., 1997b; Gatel, 1994; Ravindran and Hendriks, 2004; Adedokun et al., 2007). Also, increased intake levels of fibre and ANF increases the IEAL (Parsons et al., 1983a; Raharjo and Farrell, 1984).

2.3.2.1 ILEAL ENDOGENOUS AA LOSSES

Endogenous losses can be divided into two fractions, the basal or non-specific losses and dietary or specific losses (Lemme et al., 2004). The basal losses represent the AA losses which are influenced by dry matter intake of the animal, the animal's age and the physiological status, as well as, by experimental conditions (Ravindran and Hendriks, 2004; Stein et al., 2007b). Whereas, the specific losses represent AA losses which are influenced by the characteristics of a specific feed ingredient, such as protein, concentration and type of fibre and ANF (Moughan, 2003; Lemme et al., 2004; Stein et al, 2007b). Thus, it can be stated that the IEAL are the part of animal's requirement and therefore, AID values should be corrected for IEAL to account for accurate digestibility estimates of a feed ingredient.

When the AID coefficients of AA are corrected for basal IEAL or specific IEAL they are called standardized ileal digestibility (SID) coefficients or true ileal digestibility (TID) coefficients, respectively. The methods available to measure IEAL are: classical methods (nitrogen-free diet, regression analysis or fasted birds), peptide alimentation ultrafiltration method (enzymically hydrolysed casein), isotope dilution method and the

homoarginine method. These methods have been discussed in detail elsewhere (Sibbald, 1987; Nyachoti et al., 1997; Ravindran and Bryden, 1999). The TID is a fundamental property of the diet and is independent of dietary conditions and therefore AA digestibility in a feed ingredient should actually be expressed in terms of the TID. But, the methods used to determine specific IEAL are expensive, time consuming, have large estimation errors and are not easy to conduct routinely. In addition, for use of the TID in feed formulation the AID and IEAL are required to be determined in the same assay (Lemme et al., 2004). On the other hand, the SID coefficients are insignificantly variable with assay conditions (Ravindran and Bryden, 1999; Stein et al., 2001), and therefore can be calculated from the published estimates of basal IEAL (Lemme et al., 2004). Therefore, the SID coefficients are preferred over the TID coefficients.

Protein content of casein diets and the enzymically hydrolysed casein diets can increase the levels of IEAL (Ravindran and Hendriks, 2004; Adedokun et al., 2007; Golian et al., 2008), thus among the methods used to measure the basal IEAL, the use of nitrogen-free diet (NFD) has been suggested as the method of choice (Nyachoti et al., 1997; Ravindran and Bryden, 1999; Ravindran and Hendriks, 2004; Adedoun et al., 2007; Golian et al., 2008). The NFD method is based on the assumption that the assay diet (nitrogen-free) enables normal digestive processes to take place, and the excreted AA are of endogenous origin (Lemme et al., 2004). In the current study, IEAL values determined previously in our lab (Golian et al., 2008) using NFD method were used to calculate SID.

2.3.3 Age of birds

Research has shown that digestive capabilities in chicks are lower during the 1st week of age (Noy and Sklan, 1995; Batal and Parsons, 2002). Also, digestibility coefficients have been observed to increase with the age of birds. For instance, digestibility coefficients in 29-day old birds were observed to be lower than in 43-day old (Ten Doeschate et al., 1993). Huang et al. (2005) observed an increase in AA digestibilities in broilers from 14- to 28-day of age. The variations in Ileal endogenous amino acid losses (IEAL) due to the age and type of bird were thought to be partly responsible for the above observations (Johnson, 1992; Ravindran and Hendriks, 2004; Adedokun et al., 2007; Golian et al., 2008). For instance, Adedokun et al. (2007) reported that the IEAA flow in broilers on day 5 was double the IEAA flow on day 21. Siriwan et al. (1993) observed that the AID coefficients in isonitrogenous diet were lower in broilers (0.84) than in cockerels (0.88) (Siriwan et al., 1993). They further reported that even after correcting the AID values for IEAL, the digestibility differences remained lower in broilers than cockerels (0.90 & 0.92). From these studies it can be inferred that it is not wise to apply digestibility values estimated in adult cockerels to young broilers.

In conclusion, there exist large variations in AID of protein and AA between feedstuffs as well as among samples of the same feedstuff. These variations are attributed to differences in assay conditions/methodological factors (method of digesta collection, method of digestibility determination) or inherent feed factors (variety of grain, fibre levels, anti-nutritional values, processing conditions, fertiliser application and

environmental conditions) (Sauer and Ozimek, 1986; Knabe et al., 1989; Johnson, 1992; Mosenthin et al., 2000; Bandegan, 2009, Bandegan et al., 2009, 2010). But separate contribution of these variations (i.e. variations due to feed factors or due to assay conditions) to total variation is needed for a clear understanding of observed CV.

2.4 STANDARDIZED ILEAL DIGESTIBILITY

Additivity is an important issue in feed formulation, because it indicates whether digestible AA content of mixed diet could be predicted from the digestible AA contents of single feed ingredients. Angkanaporn et al. (1996) showed that AID of AA in soybean meal, sunflower meal, meat and bone meal are additive when combined together to form a complete diet. However, as mentioned earlier, apparent digestible values are influenced by feed intake and dietary proteins or AA contents of feed ingredients, particularly of poorly digestible and low protein ingredients (Beilorai and Iosif, 1987; Fan et al. 1994; Angkanaporn et al. 1997; Fan and Sauer, 1999), therefore, the additive nature of AID is questionable (Furuyu and Kaji, 1991; Fan et al., 1994; Stein et al., 2005). On the other hand, SID estimates are determined by allowing the bird's normal feed intake behaviour, are independent of the dietary protein levels and of the method by which AID values are originally estimated; therefore, SID estimates are more additive in a mixture of feed ingredients (Lemme et al., 2004; Stein et al., 2005, 2007a, 2007b). The AID values are corrected to SID values by accounting for basal IEAL according to the following formula:

SID, % = AID + [(basal IEAL/ AA_{diet}) × 100] where, basal IEAL = basal ileal endogenous AA losses, AA_{diet} = amino acid content of the diet.

2.5 IDEAL CHICK PROTEIN CONCEPT

The AA requirements of broilers, are influenced by many different factors, including, dietary factors (protein level, energy level, and amount of feed intake), environmental factors (disease, crowding, feeder space and heat stress), and genetic factors (sex and capacity for lean vs. fat growth) (D'Mello, 2003). However, it is neither possible to apply the AA requirements determined under certain circumstances to others, nor to consider all the factors together while determining the AA requirements (Baker and Han, 1994; Baker, 1997; Mack et al., 1999; Baker et al., 2002). This problem was addressed by poultry nutritionists by using the ideal chick protein (ICP) concept (Han and Baker, 1994; Baker, 1997) as a basis of poultry feed formulation. The ICP is defined as the blend of indispensable AA supplied in the diet fed to the bird without any excesses or deficiencies (Emmert and Baker, 1997).

The ICP concept was based on certain assumptions. First, the changes in AA requirements would not lead to changes in relative proportion of different AA. Second, the dietary requirement for each of the indispensable AA can be expressed in fixed proportion to the requirements of a reference AA as well as to the requirement for other indispensable AA. Third, the supplied AA satisfies the requirement of a bird for AA and are not utilized for protein accretion and maintenance (Emmert and Baker, 1997). The

advantage of applying the ICP concept in practical feeding is that once ideal AA ratios are once established for a certain age period, from accurately determined requirement of the reference AA under a range of different circumstances one could calculate the requirements of other indispensable AA under the same conditions (Baker, 1997). Also, if AA in broiler diets are balanced according to ICP, the increase in performance of birds would be in direct proportion to the increase in supply of AA (Vieira et al., 2004).

Although in practical broiler diets methionine is the most limiting AA, lysine was chosen as a reference AA with the requirements for all other indispensable AA expressed as a percentage of lysine. The reasons for selecting lysine as a reference AA were: (1) it is used only for protein accretion and maintenance with no other precursor role; (2) the analysis of lysine in feedstuffs is straight-forward unlike SAA and tryptophan; (3) it is the second most limiting AA in broiler diets; (4) it is easily available in synthetic form and thus its supplementation in diets is feasible; (5) the large body of data is readily available on lysine requirement for variety of dietary, environmental and body compositional circumstances (Baker and Han, 1994; Emmert and Baker, 1997).

The ideal AA ratios were derived by conducting several individual AA requirement bioassays. These procedures resulted in three or more data sets for each indispensable AA which were used to establish relationships between lysine and other indispensable AA (Baker and Han, 1994). For the successful use of ideal protein ratios in broiler diet formulation, points to be considered, are; (1) the precise estimate of dLYS, since it is the basis for setting the requirements of all other indispensable AA; (2) the use

of the same strain, sex and age of birds as well as the same basal diet in determining the requirements for all AA; (3) the diets within and between bioassays should be kept isonitrogenous and isoenergetic; (4) the graded responses should be clear-cut to the limiting AA under test; (5) the use of objective and consistent curve-fitting procedures to predict AA requirements; (6) the use of digestible rather than the total AA values of feed ingredients (Baker and Han, 1994). In addition, the digestibility coefficients of dietary AA should be used to establish accurate ideal AA ratios because the differences in AA digestibilities among various dietary sources could lead to the differences in absorption and utilization of AA and thereby changes in proportion of AA relative to each other (Emmert and Baker, 1997). This is particularly true when crystalline AA or when alternative feed ingredients like animal protein meals are used in feed formulation. Since, SID coefficients of feed ingredients are preferred over AID and TID coefficients for practical feed formulation (Stein et al., 2005; 2007b), therefore, AA requirements should also be established using SID coefficients of AA present in assay diets.

2.6 DIFFERENT IDEAL AA PROFILES

The different ideal AA profiles available for use in broiler feed formulation are NRC (1994), Baker and Han (1994), Baker et al. (2002) and Mack et al. (1994). The NRC (1994) recommendations were established based on total AA, in 0- to 3-week old broilers fed a maize-soybean meal diet, therefore NRC (1994) ratios are not considered precise when feed ingredients of different digestibility coefficients are used in feed

formulation. In contrast, Baker and Han (1994), Baker et al. (2002) and Mack et al. (1999) established AA requirement ratios on a digestible basis. Baker and Han (1994) calculated requirement ratios using crystalline AA diets fed to chicks during the second and third week of life. The Baker et al. (2002) ratios were specified for broilers from 8- to 12-day of age. Mack et al (1999) calculated the AA ratios using 9.2 g/kg lysine (required for optimum feed efficiency) as a reference point in chicks fed low protein (172 g/kg) maize-soybean meal diets from 20- to 40- days of age. The attempt to verify the efficacy of different ideal AA profiles was made by some researchers. For instance, Dari et al. (2005) conducted two experiments using corn-soybean meal based diets (Exp.1) and corn- soybean meal-wheat bran-feather meal and meat-meal-based diets (Exp. 2) formulated either according to NRC (total AA) or Illinois ICP (IICP) (digestible AA) profile with 20% CP in 3- to 6-week old Hubbard male chicks. In both experiments, BWG and economic evaluations were better for broilers fed IICP based diets, while carcass composition and carcass yield were not significantly affected by formulation procedures. In Exp. 2, birds fed diets formulated using digestible AA basis, had improved feed efficiency compared with those fed diets formulated using total AA basis, even with low CP diets. Baker and Han (1994) reported equal performance in chicks fed NRC- or IICP-based diets. In contrast, Taherkhani et al. (2005) reported that 3-week old chicks fed NRC-based diets had significantly higher BWG and FCR in both sexes relative to chicks fed IICP-based diets. The same authors (Taherkhani et al., 2005, 2008) observed similar performance in 21- to 42-day old chicks regardless of dietary AA profile. Thus, they concluded that the ratio of threonine (relative to lysine) in IICP

estimated by Baker et al. (2002) (Thr:Lys ratio of 57 %) relative to Baker and Han (1994) and Baker (1997) (Thr:Lys ratio of 67%) is insufficient for 3-week old broilers. Baker and Han (1994) indicated that except for histidine and leucine, the ratios of AA according to IICP are lower but adequate than respective ratios suggested by NRC (1994), and, the higher ratios of histidine and isoleucine suggested by IICP compared to NRC (1994) are not required. From the above studies, it can be inferred that IICP AA ratios set by Baker and Han (1994) can serve as a good basis for formulating the dose-response diets in requirement studies.

2.7 VARIABLES IN THE DETERMINATION OF AA REQUIREMENTS

Dose-response experiments relate input or independent variables such as, nutrients (from deficient to excessive levels) with output or dependent variables or response criteria, such as, growth parameters and body composition (blood or tissue component, bone strength and immune competency) (Vedenov and Pesti, 2008). The requirement of AA in broilers has been best defined by growth data in *ad-libitum* feeding studies. The growth data is measured by BWG and FCR over a defined period of time (Baker, 1986). Several reports have indicated that AA requirements vary with the response criteria (Sibbald and Wolynetz, 1986, 1987; Rhodehutschord and Pack, 1999; Baker et al., 2002; Lumpkins et al., 2007). Live BWG, feed intake and FCR measured over the intended period of time are common response criteria used to measure AA requirements. Published literature has shown a higher AA requirement for the optimum

FCR than BWG (Han and Baker, 1993, 1994; Baker and Han, 1994; Mack et al., 1999; Baker et al., 2002; Zaghari et al., 2002; Garcia and Batal, 2005; Lumpkins et al., 2007). In contrast, similar AA requirement estimates for BWG and FCR have been reported by others (Mendonca and Jensen, 1989; Morris et al., 1992; Huyghebaert and Pack, 1996; Labadan et al., 2001; Garcia et al., 2006; Aftab and Ashraf, 2009). For profitable broiler production, in addition to growth parameters, attention should be directed towards measurement of CY and BMY as response criteria (Sibbald and Wolynetz, 1986; Hickling et al., 1990; Moran and Bilgili, 1990; Acar et al., 1991; Bilgili et al., 1992; Holsheimer and Ruesink, 1993; Renden et al., 1994). The AA requirement estimates for optimum BMY has been found to be higher than optimum BWG and FCR (Sibbald and Wolynetz, 1986; Jensen et al., 1989; Hickling et al., 1990; Moran and Bilgili, 1990; Acar et al., 1991; Bilgili et al., 1992; Holsheimer and Ruesink, 1993; Han and Baker, 1994; Renden et al., 1994;Gorman and Balnave, 1995; Kidd et al., 1998; Kerr et al., 1999; Corzo et al., 2006), hence, different response criteria should be considered when estimates for AA requirements are to be determined.

Another important consideration in the determination of AA requirements is the type of mathematical model used for analyses (Leclerq, 1998; Rhodehutschord and Pack, 1999; Baker et al., 2002). For instance, Baker et al. (2002), using quadratic regression analyses, obtained 1.08% and 1.15%, whereas, using broken line methodology, obtained 0.85% and 0.97% as the lysine requirement estimates for maximum BWG and FCR, respectively. Similarly, Dozier et al. (2009) reported 1.07% and 1.10% as estimates for

the optimum BWG and FCR using quadratic regression analysis, whereas, respective estimates using the broken-line model were 1.09% and 1.15% .

2.8 METHODS TO DETERMINE AA REQUIREMENTS

Categorically, the methods used to determine AA requirements are empirical and factorial. The factorial approach takes into account a number of factors (potential of genotype, differences between and within individuals, effect of nutrient or environment on weight gain, feed intake and carcass composition, constraints on the animal by environment and feed intake) to establish optimum economic returns (D'Mello, 2003). Because of the difficulty in integrating all the above stated factors a factorial approach is not much used. The most of the published literature has employed empirical methods in requirement studies (Han and Baker, 1991, 1993; Kerr et al., 1999; Kidd and Fancher, 2001; Corzo et al., 2002). Two commonly used empirical approaches are, the graded supplementation technique, and, the diet dilution technique. D'Mello (1982, 2003) described in detail advantages and limitations of both empirical techniques, and favoured the graded supplementation technique as the method of choice for requirement studies.

The graded supplementation technique is the most frequently used method to assess the AA requirements of growing animals (D' Mello, 1982). The general methodology for this technique is to formulate a basal diet which is adequate in all other nutrients except the nutrient under test. The graded supplements of AA (synthetic or crystalline form) under test are added to a basal diet providing dose response diets

varying from deficient to excessive levels of AA under test. All the dose-response diets are kept isocaloric and isonitrogenous by addition of vegetable oil or dextrose and glutamic acid (D'Mello, 1982, 2003). To obtain correct estimates of the required AA against a given response criteria it is recommended that; (1) a minimum of 4 levels of dose response diets should be assayed so that the data can be fitted to a descriptive response curve which could facilitate objective assessment of the AA requirement (Robbins et al., 1979); (2) graded increments should be equally spaced so that statistical analysis is easier; (3) the value of regression coefficient (R^2) should be used to define the linearity of response; (4) all performance data obtained against the graded AA levels should be interpreted carefully in order to obtain accurate optimum response (Baker, 1986).

2.9 REVIEW OF THE LITERATURE AVAILABLE ON DIGESTIBLE LYSINE REQUIREMENTS ESTIMATED IN THREE-WEEK OLD BROILERS

The NRC (1994) recommendation of lysine required for 3-week old broilers is 1.10% total lysine. Although NRC recommendations have been considered adequate for broiler performance, research has indicated that they are low for maximum production of modern broilers. Han and Baker (1991), conducted an experiment to determine the lysine requirement of fast-growing (Hubbard × Hubbard) and slow-growing (New Hampshire × Columbian) broiler chicks from 8- to 21-day of age. A corn-soybean-feather meal-based lysine-deficient diet was supplemented with L-lysine.HCL to get 10 graded levels of

dietary dLYS ranging from 0.51% to 1.41%. Requirements for dLYS were determined to be 1.01% for maximum BWG and 1.21 % for maximum FCR, irrespective of the strain. They indicated that BMY in finishers increased quadratically in response to lysine addition, and the lysine requirement for maximum BMY was almost similar to that required for maximum FCR. Vazquez and Pesti (1997) using computer generated models, predicted lysine requirement estimates for optimum BWG and FCR as 1.21% and 1.32% of the diet for 0- to 3-week old broilers. Kidd et al. (1997) fed a sorghum-peanut meal-based diet at two different levels of dietary lysine (1.10% and 1.20% of diet) to broilers from 1- to18-day of age, and observed that increasing dietary lysine from 1.10% to 1.20% significantly improved BWG and FCR. Again, Kidd et al. (1998) showed that when broilers are fed dietary lysine at levels above the NRC (1994) recommendations, they have improved growth and carcass performance. In addition, they pointed out that the breast meat accretion is dependent on levels of lysine in the starter diet and is independent of levels of lysine in the grower-finisher diet. Knowles and Southern (1998) determined the lysine requirement estimates in 14- or 15-day old Cornish rock males by the one-slope broken line methodology. The dietary lysine estimates obtained were 1.00%, 0.90% and 1.10% for the average daily BWG, the average daily feed intake and the average daily FCR, respectively. Further, these authors concluded that the lysine requirement estimates suggested by NRC (1994) for starters are adequate to obtain the maximum BWG and feed intake, but not to obtain the maximum FCR. Kidd and Fancher (2001) conducted two experiments with Ross × Ross 508 male broilers from day 1 to 21 of age to study requirements of lysine in starter chicks and the effect of lysine supplied in

starter diets on the CY. The graded increments of L-lysine used to formulate test diets contained lysine from 80% to 130% of NRC (1994) recommendations. These authors reported that dietary lysine requirement for growth performance is 107% to 111% (1.18% to 1.22 % total lysine of diet or 1.07% to 1.11% digestible lysine of diet) of NRC (1994) lysine recommendations; the dietary lysine at 100% of NRC (1994) recommendations fed from day 1 to 18 of age is optimal for growth and CY at day 41 and 42 of age. Labadan et al. (2001) conducted four experiments using four different dietary CP levels (22%, 21%, 20% and 18%) in four consecutive experiments to determine the lysine requirements in male chickens from time of hatching to 8 weeks of age. They observed that lysine requirements as a percentage of total diet for maximum BWG, BMY and FCR were 1.28%, 1.32% and 1.21% from 0- to 2-week; 1.13%, 1.21 % and NS (no significant) estimate from 2- to 4-week; 0.99%, 0.99% and 1.00% from 3- to 6-week and 0.81%, 0.81% and NS estimate from 5- to 8-week. Zaghari et al. (2002) estimated lysine requirements in male and female broilers (Arian) from 6- to 21-day post-hatch. Corn-soybean meal-based lysine-deficient diets with L-lysine levels varying from 0.85% to 1.35% were assayed. They determined that males require higher levels of digestible lysine for maximum BWG and FCR (1.075% and 1.179%, respectively) than females (1.049% and 1.149%, respectively). Garcia and Batal (2005) conducted two experiments to study the digestible lysine requirements of broilers at day 4 and day 21 post-hatch. A corn-soybean-gluten meal-basal diet was supplemented to achieve dietary digestible lysine levels of 0.88%, 0.98%, 1.08%, 1.18% and 1.28% (experiment1) and 0.78%, 0.88%, 0.98%, 1.08% and 1.08% (experiment 2). The lysine requirement estimates for

optimum gain calculated using quadratic regression analysis were 0.98% and 0.95% at day 4 and 1.01% and 0.99% at day 21, respectively. Similarly for FCR, the digestible lysine requirement estimates were calculated to be 1.08% and 0.98 % at day 4 and 1.01% and 0.94% at day-21. Garcia et al. (2006) estimated that the digestible lysine requirement of 21-day old male broilers for maximum BWG are slightly higher than that of females reared in battery cages (0.96% vs. 0.94%) or floor cages (0.98% vs. 0.93%). However, based on FCR, the average dLYS requirement for females was slightly higher than that of males reared in battery (0.99% vs.0.96%) and floor pens (1.01% vs. 0.99%). Dozier et al. (2009), using the quadratic broken line methodology estimated that dLYS requirement for males and females Ross × Ross TP16 broilers was 1.10% and 1.00% of diet, respectively, for maximum BWG and FCR.

From the above stated lysine requirement studies it can be concluded that AA requirement is not a single clearly defined point. It is variable depending upon the age, sex and genotype of a bird, response criteria used, mathematical method or statistical approach applied, feed ingredient and its nutritive value and environmental factors. Therefore, recommendations established under one condition might not be optimum under other conditions.

2.10 REVIEW OF THE LITERATURE AVAILABLE ON DIGESTIBLE SULFUR AA (MET+CYS) REQUIREMENTS ESTIMATED IN THREE-WEEK OLD BROILERS

Methionine is the first limiting AA in typical corn-soybean meal- based broiler diets. It is an essential component required for protein synthesis, as a methyl donor and as a precursor for cysteine. Although physiological requirement exists for both methionine and cysteine (Graber and Baker, 1971), cysteine is a dispensable AA whose requirement is met from methionine. Therefore, both methionine and cysteine are addressed together as SAA. Considerable research has been conducted to quantify the maximum need of a bird for SAA. The NRC (1994) recommended levels of methionine for 21-day old broilers is 0.50% and for SAA is 0.90%, still most researchers and commercial nutritionists tend to use slightly higher SAA (NRC, 1994). For instance, Gormon and Balnave (1995) conducted an experiment in 21-day old Australian broilers and estimated that the percentage of dietary methionine required for optimum BWG is 0.65%. Knowles and Southern (1998) obtained the requirement estimates of 0.66% dSAA for maximum BWG and 0.63% for FCR in 15-day old broilers. Sklan and Noy (2003) estimated a requirement of 0.81% and 0.81% dietary dSAA based on BWG and FCR, respectively, for 7- day old broiler chicks. Kalinowski et al. (2003) studied the dose response relationships of methionine at fixed dietary cysteine levels and of cysteine at fixed dietary methionine levels in fast and slow feathering broilers. Using corn-soybean meal-based SAA-deficient diets formulated according to NRC (1994), and using regression equations for data analysis, these authors determined that the methionine requirement of 0.50% is adequate for 3-week old broilers regardless of feathering rate, but the cysteine requirement for slow feathering males (0.39%) is less than that required for fast feathering males (0.44%). Garcia and Batal (2005) conducted two experiments to study

the changes in dSAA requirements of broilers during the first 21-days post-hatch. Corn-soybean meal-corn gluten meal-based SAA-deficient diets were formulated to meet the NRC (1994) requirements. Graded levels of DL-met and L-cys were added to achieve dietary digestible levels of 0.68%, 0.78%, 0.88%, 0.98% and 1.08 % SAA (experiment 1) or dietary digestible levels of 0.61%, 0.71%, 0.81%, 0.91% and 1.01% SAA (experiment 2). Using quadratic regression analysis, the estimated dSAA requirements for BWG at day 4 and day 21 varied from 0.83% to 0.88% (experiment 1) and from 0.71% to 0.75% (experiment 2). The dSAA requirements for optimum FCR were 0.88% at day 4 and 0.83% at day 21 in experiment 1, and 0.81% both at day 4 and day 21 in experiment 2. Lumpkins et al. (2007) conducted three experiments with Cobb 500 chicks to evaluate variations in the estimated dSAA requirements of broilers due to rearing environment, sex, or growth performance. The SAA deficient corn-soybean meal-corn gluten meal based-diets with graded levels of dSAA ranging from 0.54% to 0.94% in experiment 1, 0.53% to 1.03% in experiment 2, 0.49% to 0.89% in experiment 3 were formulated. The dSAA requirements for maximum FCR (0.71%) and BWG (0.67%) were similar based on sex, rearing environment, or both. Whereas, due to battery and floor pens, the dSAA requirement based on maximum FCR (0.68%) was higher than the requirement estimates for maximal BWG (0.61%). Aftab and Ashraf (2009) conducted an experiment to determine SAA requirements in Hubbard × Hubbard chicks from 4- to 21-day post-hatch. Corn-soybean meal-based SAA deficient diets were formulated. The DL-methionine was supplemented to provide SAA levels ranging from 0.64% to 0.89%. The dSAA requirements were estimated to be 0.67% and 0.65% respectively, for BWG and

FCR. Schutte and Pack (1995b) reported that the requirement for SAA was 0.88% of the total lysine (or 0.75% apparent dSAA or 0.78% true dSAA) in broilers from 14 to 38 days of age.

As previously stated for lysine requirement studies, a similar conclusion can be drawn from SAA requirement studies that AA requirements vary due to bird, methodological and environmental factors. The AA requirements should be established depending upon the prevailing conditions and then used in practical feed formulation.

Since, the digestible AA system is a better representative of AA availability than the total AA system; the AA requirements should also be established using the digestible system. Moreover, genetic selection has led to production of modern birds which differ from their ancestors not only in growth rate but also in dietary requirements; thus the requirement of AA for modern broilers need to be re-established using the digestible ideal AA ratios.

2.11 CONCLUSION

The determination of digestibility coefficients of protein and AA is a reliable and practical method of determining bioavailability of proteins and AA in the diet. Further, digestibility coefficients obtained from the ileal digesta rather than excreta are a more precise indicator sof broiler performance. Since AID values are influenced by the IEAL; SID values should be estimated and used in practical broiler feed formulation. In

addition, the CV and its components associated with the digestibility estimates should also be reported to account for variations occurring due to feed or assay factors.

Methionine and lysine are the first and the second most limiting AA in broiler feeding, therefore these AA need to be supplemented in required amounts for optimum broiler production. The ideal protein/AA ratios have been proposed to exactly match the bird's requirement. Accordingly, the lysine is a reference AA and basis for calculating requirements of all other AA. Therefore, accurate estimation of the lysine requirement in a practical diet is critical. The graded supplementation technique has been considered as the most commonly used empirical method of deriving AA requirements. The published data available on AA requirement indicates that the response criteria and mathematical approaches used could considerably vary from the derived AA recommendations.

This research project aimed to estimate the SID of protein and AA in three Canadian broiler feed ingredients and to create a data set which could assist nutritionists and the broiler feed industry to achieve more accurate feed formulations. The use of SID estimates in feed formulation narrows safety margins, decreases feed cost, reduces nitrogen excretion and therefore environmental pollution. This project also aimed to derive, based on digestible ideal AA ratios, the requirements of lysine and sulphur AA for optimum growth and carcass performance of modern broilers which could maximize profitability in broiler market.

3.0 THESIS OBJECTIVES

1. To determine the AID and the SID of protein and AA in PPI, wcDDGS and corn samples fed to 3-week old broilers.

2. To derive the requirements of dlys and dSAA in 3-week old broilers fed diets formulated on the basis of IICP.

4.0 MANUSCRIPT I

STANDARDIZED ILEAL AMINO ACID DIGESTIBILITY IN PEA PROTEIN ISOLATES, DISTILLERS' DRIED GRAINS WITH SOLUBLES DERIVED FROM A BLEND OF WHEAT AND CORN AND CORN FED TO BROILERS

4.1 ABSTRACT

Three separate experiments (Exp.) were conducted with broilers to estimate the ileal digestibility of AA in four samples of pea protein isolates (PPI) (Exp. 1), five samples of distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) (Exp. 2) and five samples of corn (Exp. 3), respectively. One hundred and eighty day old Ross 308 male broiler chicks were used in Exp.1 and 216 in each of Exp. 2 and 3. The birds were fed a commercial starter diet from day 1 to day 15, followed by test diets from day 15 to day 21 of age. The four test diets in Exp.1 contained PPI, five test diets in Exp. 2 contained a mixture of wcDDGS and wheat (AC Barrie) and five test diets in Exp. 3 contained a mixture of corn and wheat, as the source of protein. Whereas, the direct method was used in Exp. 1, the indirect method was used in Exp. 2 and 3, to determine the digestibility coefficients. A sample of wheat, as a reference to test the repeatability of the digestibility experiments was also assayed separately in each Exp. All test diets were balanced for minerals and vitamins, and chromic oxide (0.3%) was included in all diets as a digestibility marker. Each test diet was randomly assigned to 6

replicate cages (each cage with 6 birds). On day 21, all birds were sacrificed to collect ileal digesta, which was analyzed to determine AID of AA. The AID estimates were then corrected for basal ileal endogenous AA losses (previously determined in our laboratory), to calculate SID of AA. The overall SID estimates for CP and AA were 92.8% and 92.4% in PPI, 67.0% and 71.5% in wcDDGS and 85.4% and 88.4% in corn, respectively. Among the indispensable AA, the SID estimates were highest for phenylalanine (96.1%), arginine (95.9%) and lysine (95.7%) in PPI, for leucine (81.1%), methionine (80.9%) and phenylalanine (80.8%) in wcDDGS, and for arginine (98.7%), methionine (97.6%) and phenylalanine (96.6%) in corn. Whereas, least digestible SID estimates were for SAA (87.2 %) in PPI, lysine (53.6 %) in wcDDGS and threonine (84.9 %) in corn, respectively. The highest CV% associated with SID estimates of AA were observed for cysteine (11.6%) and SAA (6.0%) in PPI, for lysine (27.9%) and aspartic acid (20.1%) in wcDDGS and for threonine (9.5%) and glycine (9.2%) in corn. Both the AID and the SID estimates for CP and for each AA in reference wheat were not significantly different ($P > 0.05$) across the three experiments.

4.2 INTRODUCTION

Canada is the world's largest producer of Peas (23% of total production) and Manitoba is the third largest pea producing province in Canada. Peas have high starch content and are thus an excellent source of energy in broiler feeding (Igbasan and Guenter, 1996; Igbasan et al., 1997). With low levels of methionine (3.0%) and high levels of lysine (6.5%), theAA profile of pea complements nutritionally well with canola

meal and cereals, which are high in methionine (4.5%) and low in lysine (3.0%) (NRC, 1994). Peas are known for high nutritive value and large production in Western Canada, but the chemical and nutritional composition of available peas varies among cultures (Igbasan et al., 1997; Fan and Sauer, 1999; Grosjean et al., 2000; Friesen et al., 2006), location and growing conditions (Guegen and Barbot, 1988; Igbasan et al., 1996; Fan and Sauer, 1999). Also, protein and AA digestibility (apparent and true) of peas is influenced by the composition of pea proteins (albumins or globulins), pea carbohydrates (soluble or insoluble fibre) and ANF like trypsin inhibitors, lectins, saponins and tannins (Gatel, 1994; Fan and Sauer, 1999; Grosjean et al., 2000; Wiseman et al., 2003; Gabriel et al., 2008b). Le Guen et al. (1995) observed that piglets fed diets based on PPI (derived from 2 cultures namely, Frijuanae and Finale), showed higher apparent ileal nitrogen digestibility (83.7% and 85.4% for Frijuanae and Finale, respectively) and weight gains than those fed whole pea-based diets (AID of nitrogen was 69.0% for Frijuane and 75.0% for Finale, respectively). These authors concluded that the high digestibility of PPI-based diets, compared to whole pea-based diets, is due to negligible amounts of albumins, carbohydrates and ANF in PPI. Also, it has been suggested that isolated pea protein might have modified physical properties and more accessibility to digestive enzymes (Gatel, 1994). The results of *in vitro* digestibility experiments also indicated the potential of protein isolates as an excellent protein ingredient in poultry diets. For instance, protein digestibility estimated using pepsin-trypsin and pepsin-pancreatin enzymes, was reported to be 80.6% to 82.6% and 78.6% to 79.2 % in PPI (Chavan et al., 2001), 90.0% in flaxseed protein isolates (Wanasundra and Shahiddi, 1997) and 84.0% in PPI using the multi-enzyme system (Johnson and Brekke, 1983), respectively. While

there exist studies reporting apparent digestibility of AA in PPI for piglets (Le Guen et al., 1995), in pea protein concentrates for piglets (Valencia et al., 2008) and for broilers (Valencia et al., 2009), no information is available on AID and SID of CP and AA in PPI for broilers.

In recent years, increased use of ethanol as a biofuel, has led to large availability of DDGS. Increased availability of DDGS has opened an outlet for its use in monogastric feeding, and therefore, further research related to utilization of DDGS in poultry nutrition. Various studies have demonstrated that, if broiler diets are kept nutritionally adequate in energy and lysine, DDGS can be incorporated at modest levels into poultry feeding without deleterious effects on bird performance (Runnels, 1966, 1968; Waldroup et al., 1981; Parsons et al., 1983b, Lumpkins et al., 2004; Thacker and Widyaratne, 2007; Wang et al., 2007; Youssef et al., 2008). Moreover, in modern ethanol plants, cereal feedstock is exposed to more controlled processes of heating and drying (Świątkiewicz and Koreleski, 2008), thereby yielding more valuable DDGS (often referred to as new generation DDGS) than produced from either old ethanol plants or the beverage industry (old generation DDGS). For instance, Lumpkins and Batal (2005) observed that lysine digestibility (75 to 80%) in DDGS obtained from modern beverage plants is equal to digestibility of lysine in corn (81%). Also, Lumpkins et al. (2004) has indicated that new generation DDGS is a highly acceptable feed ingredient in commercial broiler diets, which can be safely used at 6% in the starter diets and 12 to 15% in the grower-finisher diets. However, the use of DDGS as a feedstuff is of great concern since factors related to its production (drying versus wet process, original grain composition used as cereal base,

amount of solubles added back) cause large variations in its nutrient content and influences its digestibility (Martinez-Amezcua et al., 2007). While no significant variation in AA digestibilities (except for lysine) among batches of wheat-derived DDGS from the same source were observed by Bandegan et al. (2009), others reported large variations both within a source, as well as between plants (Speihs et al., 2002; Nyachoti et al., 2005; Feine et al., 2006; Lan et al., 2008).

A plethora of literature is available on the use and digestibility of old generation wheat derived DDGS in pigs (Nyachoti et al., 2005; Lan et al., 2008) and poultry (Thacker and Widyaratne, 2007; Bandegan et al., 2009) as well as of corn-derived DDGS in pigs (Stein et al., 2006; Urriola et al., 2009). Also, the AA profile and digestibility, of new generation corn-derived DDGS has been studied *in vivo* in pigs (Speihs et al., 2002; Fastinger and Mahan, 2006) and cecectomised roosters (Lumpkins et al., 2004; Lumpkins and Batal, 2005; Fastinger et al, 2006; Batal and Dale, 2006) and *in vitro* using the immobilized digestibility enzyme assay technique (Fiene et al., 2006). Thus far there are no reports on SID of protein and AA in DDGS produced from modern ethanol plants which used blends of corn and wheat as a fermentable cereal base. In addition, little information is available on the variation factors (feed or bird) associated with overall CV observed in the digestibility of AA in DDGS samples.

Because the high starch content (source of energy) and low concentration of non-soluble polysaccharides and ANF (Coweison et al., 2005), corn is the most widely used cereal in poultry diets. It was thought to be the most consistent cereal in terms of nutrient value, but this perception has been questioned by many (Sauer et al., 1977; Green et al.,

1986; Furuyu and Kaji et al., 1991; NRC, 1994; Jondreville et al., 2001; Ravindran et al., 1999; Song et al., 2004; Adedokun et al., 2008, 2009) by speculating on the variability in nutritive composition and consequently AA digestibility coefficients in corn. It has also been demonstrated that field location and corn heat unit (CHU) rating of corn cultivars could also affect the nutrient composition and agronomic parameters of corn (Opapeju et al., 2007). Enormous production of high-yielding, low corn heat units (CHU)-rated cultivars in Manitoba has increased the potential of corn for use in broiler feeding. But, to date, digestibilities of AA in Manitoba-grown corn for broilers have not been determined. Therefore, it is critical to evaluate CP and AA digestibilities and their associated variability among corn grown in different parts of Manitoba before utilizing them in feed formulation.

With respect to AA nutrition, the nutritive value of a feed ingredient is judged more accurately by AA availability than AA profile. The best and most practical indicator of AA availability is the AA digestibility. There is no doubt that ileal rather than fecal digesta is a better predictor of AA digestibility in poultry, but AA digestibility is influenced by genotype, age, sex and method of determination (Ten Doeschate et al., 1993; Song et al., 2004; Huang et al., 2005; Garcia et al., 2007). The AID values are dependent on AA/protein intake,does not take into account the endogenous losses in digesta and therefore are non-additive in mixed diets and AID values needs correction before use as a reference in feed formulation. In commercial feeding both high quality (high digestibility) and low quality feed ingredients (low digestibility) are utilized in feed formulation, therefore it is crucial that digestibility estimates of different feed ingredients

are additive when used in mixed diets. The AID values corrected for basal IEAL produces SID values which are additive in mixtures of feed ingredients and a closer representative of true digestibility (Stein et al., 2005; 2007a).

4.3 OBJECTIVES

To determine the AID and SID estimates of protein and AA and their associated CV in PPI, wcDDGS and corn samples fed to 21-day old broilers.

4.4 MATERIALS AND METHODS

4.4.1 DIETS

Four PPI (from Nutri-pea limited at Portage La Prairie, Manitoba, Canada), five wcDDGS (from Husky's Mohawk Ethanol plant at Minnedosa, Manitoba, Canada) and five corn (from different locations in Manitoba, Canada) samples were assayed in three independent experiments. The PPI samples were collected from five different processing periods; wcDDGS samples were collected from five different fermentation batches with different grain source and processing days; and corn samples were obtained from five different local farms. A sample of reference wheat (AC Barrie) was also obtained from a local farm. Each test diet in Exp 1 contained PPI (25%) as the sole source of protein while 51% and 47.7% of wheat was mixed with wcDDGS in Exp. 2 and with corn in Exp. 3, respectively. The reference wheat assayed in each experiment was included at

91.7%. All test diets contained chromic oxide (0.3%) as a digestibility marker and were balanced for minerals and vitamins as per NRC (1994) recommendations.

4.4.2 BIRDS AND CONDUCT OF EXPERIMENT

The experimental protocol 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 one-hundred eighty (Exp.1) or two hundred sixteen (Exp. 2 or Exp. 3) Ross 308 male broilers (Ross, Aviagen) were obtained from a local hatchery (Carlton Hatchery, Grunthal, Manitoba, Canada) and were housed in Petersime battery brooders (Petersime Incubator Co., Gettysburg, Ohio). Batteries were placed in a well-illuminated (24 h fluorescent lightning) and temperature controlled (32, 28 and 24°C during 1, 2 and 3 week, respectively) in the room. Chicks received a commercial starter diet (FeedRite, Ridley Inc., Winnipeg, Manitoba, Canada) in the form of crumbles (3100 Kcal/Kg, 19% CP) from day 1 to day 14 of age. On day 15, chicks were fasted for 3 hours, weighed and allotted in groups of 6 to 30 pens (Exp. 1) or (Exp. 2 or Exp. 3) 36 pens such that weight distribution per pen was similar. Each of five (Exp. 1) or six (Exp. 2 or Exp. 3) diets per experiment were randomly assigned to six replicate cages and fed to chicks for one week. The feed and water were offered *ad libitum* throughout the experimental period. On day 21, birds were killed by cervical dislocation and body cavity of each bird was opened.

The section between Meckel's diverticulum and 4 cm anterior to the ileo-ceco-colonic junction was isolated and contents were gently squeezed and collected into sample bags. Digesta from all the birds within a cage was pooled in a bag, immediately frozen and subsequently freeze dried. The dried ileal digesta sample bags were stored in airtight bags and stored at room temperature until analysis.

4.4.3 CHEMICAL ANALYSIS

Test diets and digesta samples were finely ground using a coffee grinder (CBG5 Smart Grind; Applica consumer products, Inc., Shelton, CT) for dry matter, nitrogen and AA analysis. Dry matter was determined according to the AOAC (2000) method (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 diet and digesta were determined by Evonik-Degussa Corporation (Hanau-Wolfang, Germany) 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 then subsequently neutralized with sodium metabisulfite (Commission Directive, 1998). Amino acid concentrations were quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. For chromium analysis, samples were ashed at 600°C for 12 hours in a muffle furnace and analyzed using inductively coupled plasma mass spectrometry (ICS-AES Vista, Varian) according to the method (procedure # 985.01) of AOAC (2005).

4.4.4 DIGESTIBILITY CALCULATIONS

The AID estimates of protein and AA in PPI (Exp. 1) and in reference wheat were calculated using the direct method (Fan and Sauer, 1995) as follows:

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

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

Because difference method is more appropriate than direct method for feedstuff which have poor palatability or have low protein content (Fan and Sauer, 1995), the AID coefficients of protein and AA in wcDDGS (Exp. 2) or corn (Exp. 3) were calculated using the difference method (Fan and Sauer, 1995). Assuming that there are no interactions between the digestibility values of AA in the basal (wheat) and test feed ingredient (wcDDGS or corn), the relationship between digestibility values can be expressed according to equation [1]. The digestibility values of AA in the test feed ingredient were then determined according to equation [2] (Van Leeuwen et al., 1987).

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

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

D_D = AID of an AA in the test diet (%);

D_B = AID 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= AID 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 (%)

Basal ileal endogenous AA losses (see Appendix, Table 9.1) estimated previously in our lab using a nitrogen-free diet (Golian et al., 2008) were used for the correction of AID values to the SID values of AA in all three experiments using the following formula:

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

Where, IAA_{end} = Ileal endogenous AA loss, and AA diet = amino acid content of the diet.

4.4.5 STATISTICAL ANALYSIS

The digestibility estimates of CP and AA from each experiment (PPI, wcDDGS, and corn and reference wheat samples) were analyzed using the Nested procedure of SAS Institute, Inc. (1990). The model used in data analysis was: $Y_{ij} = \mu + t_i + e_{ij,}$ where Y_{ij} = digestibility of j'th cage of bird in i'th sample, μ = population mean, t_i = effect of the i'th sample and e_{ij} = error deviation of the j'th AA in the i'th treatment. The variance due to the samples (σ_s^2) and cages within samples (σ_e^2) was determined for AID and SID for CP

and for each AA (in each experiment). These variance measurements used to calculate total coefficients of variation (CV, %) showed the relative importance of between sample variability and within sample variability.

4.5 RESULTS AND DISCUSSION

Crude protein and AA concentration in PPI, wcDDGS, corn samples are presented in Table 4.1, also, the protein and AA contents in the reference wheat are included in footnotes. The protein and AA content in PPI was higher than published values of CP and AA in peas (Igbasan et al., 1997; Ravindran et al., 2005; Bandegan, 2009; Owusu-Aseidu et al., 2002) but compares well with the AA ranges published previously for pea protein (Jondreville et al., 2001; Degussa, 2006). This study demonstrated that PPI have high amounts of leucine (6.5%) and lysine (5.6%) among indispensable AA and glutamic acid (14.3%) and aspartic acid (8.9%) among dispensable AA but low amounts of histidine (1.9%) and SAA (1.6%), methionine being the lowest (0.8%). Among the indispensable AA, similar trends were reported previously (Chavan et al., 2001). Earlier reports of high contents of lysine and low contents of SAA in peas (Gatel, 1994; NRC, 1994; Bandegan, 2009) have been explained by the AA composition of storage protein which are high in lysine but low in SAA (Croy et al., 1980). Globulins rather than albumins contain higher amounts of arginine and dispensable AA, and these AA get further concentrated when pea protein is separated from starch (Igbasan et al., 1997). While the most variable AA in PPI were isoleucine and valine with CV of 14.4% and 12.9%, methionine and SAA were least variable among indispensable AA with CV

Table 4.1. Crude protein and AA composition (%) in pea protein isolate (PPI) (n = 4), distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) (n = 5) and corn (n = 5) fed to broilers[1,2] (DM basis)

Items	PPI			wcDDGS			Corn		
	Range	Mean	CV%[3]	Range	Mean	CV%	Range	Mean	CV%
DM	94.6 - 95.3	94.9	0.30	92.8 - 93.5	93.1	0.40	88.0 - 90.5	89.9	0.90
CP	84.4 - 86.0	84.9	0.89	30.7 - 31.9	31.4	1.45	6.22 - 7.51	6.77	8.06
Indispensable AA									
Arg	5.85 - 7.15	6.40	8.60	1.17 - 1.27	1.23	2.93	0.30 - 0.38	0.34	9.50
His	1.76 - 2.14	1.91	8.99	0.89 - 0.94	0.92	2.15	0.20 - 0.28	0.23	11.8
Ile	2.98 - 4.18	3.51	14.4	1.36 - 1.44	1.40	2.80	0.25 - 0.32	0.29	9.88
Leu	6.19 - 7.14	6.52	6.84	3.61 - 3.95	3.76	3.65	1.01 - 1.18	1.12	9.21
Lys	5.34 - 6.11	5.64	5.92	0.79 - 0 .85	0.82	2.65	0.20 - 0.26	0.23	9.06
Met	0.77 - 0.82	0.80	2.57	0.71 - 0.79	0.75	4.13	0.18 - 0.28	0.21	18.0
TSAA	1.54 - 1.66	1.58	3.27	1.35 - 1.47	1.42	3.63	0.34 - 0.48	0.40	15.7
Phe	3.98 - 4.53	4.16	6.39	1.58 - 1.61	1.60	0.74	0.37 - 0.47	0.42	11.9
Thr	2.54 - 2.85	2.68	4.83	1.11 - 1.22	1.16	3.12	0.28 - 0.39	0.35	13.2
Val	3.04 - 4.10	3.52	12.93	1.53 - 1.59	1.57	1.41	0.31 - 0.36	0.34	5.74
Dispensable AA									
Ala	3.28 - 3.54	3.42	3.52	2.07 - 2.27	2.15	3.85	057 - 0.75	0.66	14.2
Asp	8.35 - 9.54	8.90	5.45	1.89 - 1.98	1.96	1.79	0.47 - 0.62	0.55	12.5
Cys	0.72 - 0.85	0.78	7.33	0.64 - 0.71	0.67	3.83	0.15 - 0.24	0.19	19.2
Glu	13.5 - 14.9	14.3	4.28	6.43 - 7.24	6.84	4.43	1.44 - 1.94	1.69	14.4
Gly	2.67 - 3.22	2.88	8.34	1.21 - 1.25	1.23	1.49	0.25 - 0.30	0.28	8.24
Pro	3.08 - 3.43	3.25	4.41	2.64 - 2.79	2.73	2.15	0.66 - 0.83	0.77	12.9
Ser	3.53 - 3.97	3.75	4.90	2.39 - 1.52	1.44	3.48	0.32 - 0.46	0.40	15.3

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

2. The values of DM, CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser in reference wheat sample were 94.9, 15.3, 0.59, 0.34, 0.40, 0.98, 0.38 , 0.31, 0.67, 0.72, 0.49, 0.50, 0.58, 0.74, 0.36, 4.96, 0.59, 1.58 and 0.69, respectively.

3. CV = Coefficient of variations between different samples of same ingredients.

of 2.6% and 3.3%. The nutrient variability observed among the PPI samples assayed in current study could be explained by publishes reports about cultures, location and growing conditions (Guegen and Barbot, 1988; Igbasan et al., 1996; Fan and Sauer, 1999) which tend to vary the chemical and nutritional composition of whole-peas.With some differences in concentrations of arginine and valine, CP and AA concentrations of wheat compare well with wheat assayed previously in our lab (Bandegan et al., 2009) and also with those reported earlier (Thacker and Widyaratne, 2007; Lan et al., 2008). The CP and AA concentrations of corn samples were within the range reported in previous studies (NRC, 1994; Ravindran et al., 1999; Opapeju et al., 2007) but were different from others (Garcia et al., 2007; Adedokun et al., 2008). The variability observed in the AA composition of cereals is due to variations in genetic variety, cultivars and geographical location (where crop is grown) (Opapeju et al., 2007), husbandry practices, season and year of harvest (Evers et al., 1999; Jondreville et al., 2001).

Interestingly, the CP and AA levels in wcDDGS, were closer to values reported for corn DDGS (NRC, 1994; Speihs et al., 2002; Fastinger and Mahan, 2006; Fastinger et al., 2006; Stein et al., 2006) than for wheat DDGS (Bandegan et al., 2009), which may be due to the original grain composition used for fermentation by ethanol plants. Distiller's dried grains with solubles assayed in this study were obtained at the time when a blend of approximately 75% corn and 25% wheat was used as a cereal base for ethanol production. The protein and AA content in wcDDGS were observed to be higher than respective contents in wheat and corn samples. Studies in pigs and poultry using wheat-derived DDGS (NRC, 1994; Nyachoti et al., 2005; Thacker and Widyaratne, 2007; Lan et

al., 2008; Bandegan et al., 2009) have also reported AA content to be 2 to 3 folds higher than in wheat. This is because removal of alcohol and carbon dioxide (fermentation products) leaves behind concentrated amounts of other nutrients. The higher nutrient content than cereal base and enormous availability has encouraged the use of DDGS in poultry diets. However, attention should be directed towards the energy and lysine content of broiler diets which contain high levels of DDGS to prevent impaired feed utilization and bird performance (Waldroup et al., 1981; Parsons et al., 1983b; Lumpkins et al., 2004; Thacker and Widyaratne, 2007).

Of the entire indispensable AA assayed in wheat, wcDDGS and corn, the contents of methionine (0.31%, 0.75% and 0.21%) and lysine (0.38%, 0.82% and 0.23 %) were lowest and that of leucine (0.98%, 3.65% and 1.12 %) were highest, respectively. In terms of the variability observed for the indispensable AA profile, methionine and SAA were most variable both in wcDDGS and corn, while phenylalanine in wcDDGS and valine in corn were least variable. A comparison of protein and AA variability revealed corn as the most variable and wcDDGS as least variable among the feed ingredients analyzed. The high CV observed among the corn samples in current study could be due to differences in location or cultivars as has been previously demonstrated by Opapeju et al. (2007). The corn samples assayed by Opapeju et al. (2007) were also obtained from different parts of Manitoba.

The AID and SID of CP and AA in PPI, wcDDGS, corn and reference wheat (included in footnote) are shown in Tables 4.2 and 4.3. The average AID and SID estimates of indispensable AA in PPI ranged from 82.5% for SAA to 94.7% for arginine,

Table 4.2. Apparent ileal protein and AA digestibility coefficients in pea protein isolate (PPI) (n = 4), distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) (n = 5) and corn (n = 5)[1,2] samples fed to broilers.

Items	Pea protein isolates		wcDDGS		Corn	
	Range	Mean	Range	Mean	Range	Mean
CP	85.8 - 94.0	90.6	61.2 - 70.1	65.1	77.9 - 86.2	82.1
Indispensable AA						
Arg	89.2 - 96.8	94.7	71.3 - 79.8	75.4	92.7 - 99.9	95.3
His	85.5 - 94.6	92.5	60.1 - 73.2	67.7	88.8 - 93.7	90.7
Ile	85.5 - 93.5	90.7	63.8- 72.2	68.0	85.7 - 92.5	88.4
Leu	87.6 - 95.0	92.0	76.5 - 82.1	79.8	92.3 - 95.8	93.8
Lys	90.9 - 96.8	94.5	44.3 - 59.4	50.7	84.1 - 92.0	87.6
Met	86.3 - 95.1	90.8	76.7 - 83.1	79.6	93.6 - 97.1	95.2
Met+Cys	75.5 - 87.9	82.5	69.0 - 75.9	72.8	88.0 - 92.8	90.2
Phe	87.7 - 95.0	92.2	74.6 - 80.3	77.2	88.1 - 93.0	89.8
Thr	77.8 - 90.6	86.0	56.0 - 63.5	59.0	69.8 - 80.0	73.9
Val	83.9 - 93.2	89.1	63.3 - 71.5	67.4	85.5 - 92.4	88.1
Dispensable AA						
Ala	84.2 - 94.0	89.6	71.7 - 79.8	76.1	90.9 - 95.0	92.8
Asp	87.6 - 94.6	91.9	50.1 - 61.0	54.7	84.6 - 92.2	87.7
Cys	58.9 - 82.5	72.6	59.5 - 67.2	62.6	81.2 - 87.8	83.5
Glu	90.3 - 96.9	94.6	77.8 - 82.9	80.2	91.8 - 96.9	94.4
Gly	78.4 - 92.6	88.1	55.5 - 64.5	59.2	79.5 - 89.3	83.0
Pro	83.1 - 91.8	89.2	73.5 - 80.8	77.1	86.9 - 92.2	89.7
Ser	82.7 - 92.9	88.9	64.2 - 70.8	67.2	82.3 - 89.2	84.8

1. n = number of different samples of a feed fed to 6 cages, each with 6 birds.
The average AID coefficients in reference wheat samples of CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser were 87.8, 83.1, 87.1, 89.4, 89.5, 81.2, 91.9, 91.1, 93.1, 83.8, 86.2, 82.5, 82.0, 90.1, 95.8, 83.6, 95.1 and 88.1, respectively.

Table 4.3. Standardized ileal protein and AA digestibility coefficients in pea protein isolate (PPI) (n = 4), distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) (n = 5) and corn (n = 5)[1,2] samples fed to broilers[3]

Items	PPI		wcDDGS		Corn	
	Range	Mean	Range	Mean	Range	Mean
CP	88.1 - 96.1	92.8	63.2 - 72.1	67.0	81.1 - 89.6	85.4
Indispensable AA						
Arg	90.5 - 98.0	95.9	73.2 - 81.8	77.3	96.7 - 103	98.7
His	87.6 - 97.9	94.4	61.8 - 74.8	69.3	91.6 - 96.6	93.6
Ile	88.0 - 96.2	92.9	66.2 - 74.6	70.3	90.2 - 97.1	92.9
Leu	89.7 - 96.7	93.9	77.9 - 83.5	81.1	93.8 - 98.6	96.5
Lys	92.3 – 98.0	95.7	47.0 - 62.3	53.6	89.0 - 97.0	92.5
Met	87.5 - 97.2	93.5	78.0 - 84.5	80.9	96.1 - 99.6	97.6
Met+Cys	80.4 - 91.8	87.2	71.2 - 78.1	74.9	91.7 - 96.6	93.9
Phe	92.2 - 98.7	96.1	78.3 - 84.0	80.8	94.9 - 99.9	96.6
Thr	84.8 - 96.7	92.3	61.1 - 69.0	64.3	80.7 - 91.2	84.9
Val	86.9 - 95.8	91.9	65.8 - 74.0	69.8	90.5 - 97.2	92.8
Dispensable AA						
Ala	83.9 - 96.5	92.2	73.5 - 81.5	77.8	94.5 - 98.6	96.3
Asp	89.7 - 96.4	93.7	53.3 - 64.2	57.8	90.6 - 98.3	93.8
Cys	66.7 - 89.1	79.7	62.4 - 70.2	65.6	86.2 - 92.7	88.5
Glu	92.0 - 98.3	96.0	78.6 - 83.8	81.0	93.3 - 98.4	96.0
Gly	81.9 - 95.6	91.2	58.0 - 67.1	61.8	84.4 - 94.2	87.9
Pro	88.1 - 96.3	92.5	74.9 - 82.1	78.3	89.3 - 94.6	92.1
Ser	86.6 - 96.2	92.3	67.3 - 74.0	70.3	88.1 - 95.2	90.7

1. n = number of different samples of a feed fed to 6 cages, each with 6 birds.
2. The average AID coefficients in reference wheat samples of CP, Arg, His, Ile, Leu, Lys, Met, Met+Cys, Phe, Thr, Val, Ala, Asp, Cys, Glu, Gly, Pro and Ser were 87.8, 83.1, 87.1, 89.4, 89.5, 81.2, 91.9, 91.1, 93.1, 83.8, 86.2, 82.5, 82.0, 90.1, 95.8, 83.6, 95.1 and 88.1, respectively.
3. The SID estimates were calculated by correcting AID estimates for IEAL determined previously in our lab (Golian et al., 2008).

and 87.2% for SAA to 96.1% for phenylalanine, respectively. Similar estimates for dispensable AA varied from 72.6% for cysteine to 89.6% for alanine and 79.7% for cystiene to 96.0% for glutamic acid. A trend similar to this for AA digestibility values was reported in protein isolates of beach pea (Chavan et al., 2001) and pea (Bandegan, 2009). The ranking of AA in terms of highest (glutamic acid) and lowest (SAA, particularly cystiene) digestibility values compares well with previous reports on SID of AA in field peas for adult cockerels (Igbasan et al., 1997), and on AID of AA in peas for cecectomized broiler chickens (Gabriel et al., 2008a). No published data was available on AID and SID of AA in PPI in broilers for comparison purposes. The AID estimates of AA in PPI fall within the range reported for AID estimates for peas in broilers (Wiseman et al., 2003; Gatel, 1994), but are higher than AID estimates published for peas using adult cockerels (Igbasan et al., 1997) and broilers (Kluth et al., 2005; Gabriel et al., 2008a). Also, our AID estimates were higher than the previously reported AID estimates for pea protein concentrates in broilers (Valencia et al., 2009). Different digestibility estimates reported by different researchers could be due to the difference in feed ingredient assayed (whole pea or pea protein isolate or pea protein concentrate), type of bird (intact or cecectomised birds or adult cockerels) or digestibility estimates (apparent or standardized or true).

Pea protein isolates assayed in this study were obtained from Nutri-pea Limited where PPI were prepared according to the wet process reviewed in detail by Gueguen (1983) from finely ground dehulled raw peas. The wet process produce PPI in the following steps: (1) NaOH alkalinisation and centrifugation of finely ground dehulled

raw peas to separate starch and fibre from protein slurry; (2) acidification of protein slurry using HCl to make protein curd; (3) neutralization of protein curd by NaOH, and (4) application of dry heat to obtain protein isolate powder. Pea protein isolated in this way accounted for 810g CP/kg (as analyzed by Nutri-pea Limited and in our laboratory). The wet process concentrates proteins only; whereas, the dry process (or air classification technique used to prepare pea protein concentrates) concentrates proteins as well as ANF (Gueguen, 1983). Removal of ANF during the process of isolating pea protein removes 90% of albumins, and leaves easily digestible globulins (Le Guen et al., 1995). The addition of pea ANF concentrate to pea protein isolate diet in pigs has been shown to reduce AID by 7 units (Le Guen et al., 1995). The non-purified fibre in raw peas, as opposed to purified fibre in PPI may lead to increased mucus production or decreased re-absorption of endogenous AA in the small intestine (De Lange et al., 1989) and decreases N and AA digestibility (Furuyu and Kaji, 1991). Moreover, dehulling and fine grinding of peas also improves AA digestibility (Igbasan et al., 1997; Valencia et al., 2008, 2009) by removing fibre content and effect of tannins and by decreasing particle size (Gabriel et al., 2008b). Therefore, the presence of ANF in pea protein concentrates and of ANF, carbohydrates, pea pectins and pea cell walls in whole raw peas accounts for their low AA digestibility (Gatel and Grosjean, 1999; Le Guen et al., 1995). In addition, heat treatment has been shown to be an effective method of increasing AA digestibility by either inactivating protease inhibitors (Stein and Bohlke, 2007) or by changing conformation of pea proteins and making them more accessible to digestive enzymes (Owusu-Aseidu et al., 2002). Since PPI is devoid of ANF, fibre and cell wall components, the digestibility of PPI is higher compared to whole-peas. Therefore, the

estimates of AA digestibility obtained in our study were higher than digestibility coefficients published previously for raw peas and pea protein concentrates (Igbasan et al., 1997; Kluth et al., 2005; Gabriel et al., 2008b; Valencia et al., 2009).

The highest AID estimate for arginine and lysine, while the lowest estimates for SAA obtained in this study and in earlier studies on peas (Gatel, 1994; NRC, 1994; Fan and Sauer, 1999; Wiseman et al., 2003; Friesen et al., 2006), PPI (Le Guen et al., 1995) and pea protein concentrates (Valencia et al., 2009) supports the hypothesis of enzyme specificity as an important indicator of apparent AA absorption in the small intestine (Fan and Sauer, 1999). Based upon known specificity of proteases and peptidases, it is assumed that arginine and lysine are absorbed first after enzymatic hydrolysis (Low, 1980). A low degree of SAA digestibility in peas has been related to the presence of ANF like protease inhibitors. The pancreatic proteases are rich in methionine and cysteine and the presence of protease inhibitors divert the SAA from body tissue synthesis to additional production of pancreatic enzymes (Gatel, 1994; Wiseman et al., 2003).

Among the indispensable AA of wheat, AID and SID estimates of methionine (89.9% and 91.9%), SAA (88.2 % and 91.1%) and phenylalanine (87.8% and 93.1%) were highest and that of threonine (74.8% and 83.8%) and lysine (77.3% and 81.2%) were lowest. These results are in close agreement with the AID coefficients reported previously in our lab for pigs (Nyachoti et al., 2005) and poultry (Bandegan et al., 2009) and those of others in broilers (Ravindran et al., 1999, 2005; Huang et al., 2005). Both in terms of AID and SID, arginine (95.3% and 98.7%) and methionine (95.2% and 97.6%) were the most digestible, while threonine was the least digestible (73.9% and 84.9%)

among indispensable AA in corn. Similarly, in wcDDGS, leucine (79.8% and 81.1%), methionine (79.6% and 80.9%) and phenylalanine (77.2% and 80.8%) had the highest estimates of AID and SID, and lysine (50.7% and 53.6 %) and threonine (59% and 64.3%) had the lowest estimates. Similar trends were reported in our previous studies using wheat DDGS in broilers (Bandegan et al., 2009) and pigs (Nyachoti et al., 2005; Lan et al., 2008). Overall, average AID and SID estimates of CP and AA in wcDDGS (69.1% and 71.5%) were lower than respective values in wheat (84.0% and 87.7%), and corn (88.8% and 93.25%). The high fibre concentration in DDGS (Nyachoti et al., 2005; Stein et al., 2006) might be responsible, in part, for the low CP and AA digestibility. In addition, heat treatment used during the drying process of DDGS could damage the protein; or the use of AA in DDGS for microbial protein synthesis during fermentation have been suggested as a possible causes of reduced digestible AA values in DDGS compared to the cereal base (Stein et al., 2006).

In the present study, lysine was found to be the most variable and least digestible AA in wcDDGS with AID and SID estimates of 50.7% and 53.6 %, respectively. This finding concurs with the earlier reports for corn DDGS in poultry (Lumpkins et al., 2004; Lumpkins and Batal, 2005; Batal and Dale, 2006; Cromwell et al., 1993; Speihs et al., 2002) as well as wheat DDGS in pigs (Nyachoti et al., 2005; Lan et al., 2008) and poultry (Thacker and Widyaratne, 2007; Bandegan et al., 2009). The lower availability of lysine and other AA in DDGS could be the result of heat damage of these AA, as observed for over-processed soybean meal (Parsons et al., 1992) and for dark coloured DDGS (Batal and Dale, 2006; Fastinger et al., 2006). At high drying temperatures maillard reactions occurs between proteins and carbohydrates. Amino acids get attached to reducing sugars

and become cell bound which protects them from digestive action of proteases, making them poorly digestible (Mauron, 1981). Furthermore, increased ileal endogenous flow of nitrogen and AA could lower respective AID values (Nyachoti et al., 1997). Threonine is poorly absorbed in the small intestine and is a major indispensable AA in gut secretions (Siriwan et al., 1993; Ravindran and Hendriks, 2004; Ravindran et al., 2005) and, therefore, was found to be among the least digestible AA in all feed ingredients assayed in the present study.

Most of the digestibility data currently available for corn has been determined either in cecectomized roosters (Sibbald, 1979; Green et al., 1987) or in grower-finisher broilers (Ravindran, 1999; Huang et al., 2005, 2006). Since it has been shown that AID coefficients varies with genotype and age of the bird (Ten Doeschate et al., 1993; Huang et al., 2006); it is difficult to compare AA digestibility values obtained in 3-week old broilers to older age group. With slight differences, the SID coefficients for corn obtained in the present study concurs with SID estimates reported previously (Lemme et al., 2004; Garcia et al., 2007; Adedokun et al., 2008). The SID estimates of arginine, methionine, alanine, aspartic acid, glutamic acid and serine in corn were higher than the respective values determined by Garcia et al. (2007). Garcia et al. (2007) used Cobb 500 and enzymatically hydrolysed casein method to obtain SID coefficients. Adedokun et al. (2008) determined SID values by using either the high density protein diet (HDP) or nitrogen free diet (NFD) in 21-day old male Ross 308 broiler chicks (similar to the strain used in the present study). The average SID values determined by HDP rather than determined by NFD (Adedokun et al., 2008) fall within the range reported in our study for SID values for all AA except isoleucine, methionine, glutamic acid and serine. The

high SID values obtained for the last four stated AA were observed to be greater than 100%, as explained by the authors (Adedokun et al., 2008).

The AID is a function of bird behaviour (or methodology) and feed ingredients composition (Ravindran et al., 2005; Huang et al., 2005, 2006; Adedokun et al., 2009); therefore, variations in feed utilization among birds and variations in nutritional composition among feed samples would contribute to variations in AA digestibilities. Utilization of feed ingredients in poultry diets with no clear understanding of variations observed will at best result in variable improvements in performance and at worst reduce the performance, uniformity and profitability of the end product. Hence, it is as important to report CV associated with digestibility coefficients as to report digestibility values.

The CV and its components associated with the AID and SID estimates of each AA in PPI, wcDDGS and corn are presented in Table 4.4 and 4.5. In all three feed ingredients, the CV values for AID estimates of AA were observed to be greater than corresponding SID estimates, emphasizing the effect of varying bird feed intake levels. Because correcting apparent digestibility to standardized digestibility to overcome problems related to intake variability, SID values should be used in practical feed formulation. Of the three feed ingredients assayed, the CV range was widest for wcDDGS (6.6% to 29.4%) and narrowest for corn (3.2% to 10.7%), with threonine and lysine as the most variable and leucine and methionine as the least variable indispensable AA. In PPI, the CV ranged from 2.7% to 13.5%, with lysine, leucine and methionine as least and threonine and SAA as the most variable indispensable AA. Studying the

Table 4.4. Coefficient of variation (CV) associated with AID of CP and AA determined in broiler chicks fed diets containing pea protein isolate (PPI) (n = 4), distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) (n = 5) and corn (n=5) samples[1]

Item	PPI			wcDDGS			Corn		
	CV%[2,3]	Variance components		CV%	Variance components		CV%	Variance components	
		Between samples	Within samples		Between samples	Within samples		Between samples	Within samples
CP	3.7	72.0	28.0	14.8	14.5	85.5	8.6	41.1	58.9
Indispensable AA									
Arg	4.4	62.7	37.3	12.3	20.9	79.1	7.0	29.2	70.8
His	4.1	69.9	30.1	18.4	24.5	75.5	5.5	12.1	87.9
Ile	3.7	65.7	34.3	12.9	14.3	85.7	6.3	41.8	58.2
Leu	3.3	69.5	30.5	7.3	15.2	84.8	3.2	33.5	66.5
Lys	2.7	69.5	30.5	29.4	24.4	75.6	7.9	32.1	67.9
Met	4.6	64.2	35.8	8.1	24.0	76.0	3.0	26.6	73.4
Met+Cys	6.8	69.7	30.3	9.8	6.4	93.6	4.4	37.7	62.3
Phe	3.2	66.4	33.6	8.6	7.7	92.3	5.6	10.6	89.4
Thr	6.1	79.1	20.9	16.7	8.3	91.7	10.7	42.9	57.1
Val	4.6	74.3	25.7	13.5	67.4	32.6	6.5	35.2	64.8
Dispensable AA									
Ala	5.4	73.0	27.0	9.6	22.4	77.6	3.9	29.9	70.1
Asp	2.9	72.1	27.9	21.1	14.8	85.2	6.8	40.0	60.0
Cys	13.5	73.1	26.9	12.1	0.0	100.0	6.7	44.1	55.9
Glu	2.7	70.8	29.2	6.6	9.5	90.5	4.0	40.7	59.3
Gly	4.8	71.9	28.1	18.6	13.5	86.5	9.7	40.4	59.6
Pro	4.5	75.8	24.2	8.8	14.8	85.2	4.5	44.8	55.2
Ser	4.7	82.0	18.0	12.0	8.9	91.1	6.6	42.9	57.1

1. Samples were assayed in 6 replicate cages (each with 6 birds).

2. Total CV = $\frac{\sqrt{\sigma e^2 + \sigma s^2}}{\bar{y}} \times 100\%$, where σs^2 = variance due to samples; σ_e^2 = variance due to cages within samples and $\bar{y}$ = respective mean of the PPI or wcDDGS or corn samples as shown in Table 4.2.

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 4.5. Coefficient of variation (CV) associated with SID of CP and AA estimates determined in broiler chicks fed diets containing pea protein isolate (PPI) (n = 4), distiller's dried grains with solubles derived from a blend of wheat and corn (wcDDGS) (n = 5) and corn (n = 5)[1] samples

Item	PPI			wcDDGS			Corn		
	CV%[2,3]	Variance components		CV%	Variance components		CV%	Variance components	
		Between samples	Within samples		Between samples	Within samples		Between samples	Within samples
CP	3.5	69.9	30.1	14.4	15.0	85.0	8.3	41.9	58.1
Indispensable AA									
Arg	2.8	60.3	39.7	12.1	21.2	78.2	6.7	29.1	70.9
His	3.9	66.7	33.3	17.9	24.2	75.8	5.4	12.9	87.1
Ile	3.4	61.1	38.9	12.6	14.8	71.1	6.0	36.8	63.2
Leu	3.1	65.8	34.2	7.2	15.2	84.8	3.2	37.3	62.7
Lys	2.5	66.1	33.9	27.9	24.7	75.3	7.5	32.6	67.4
Met	4.2	58.6	41.4	8.2	24.2	75.8	3.0	29.1	70.9
TSAA	6.0	64.8	35.2	9.5	6.7	100.0	4.3	39.2	60.8
Phe	2.8	56.7	43.3	8.4	9.1	90.9	5.4	13.0	87.0
Thr	5.1	73.2	26.8	15.6	14.6	85.4	9.5	44.3	55.7
Val	4.2	70.6	29.4	13.2	14.1	85.9	6.3	35.9	64.1
Dispensable AA									
Ala	5.0	70.0	30.0	9.4	22.1	77.9	3.9	33.5	66.5
Asp	2.7	68.1	31.9	20.1	15.3	84.7	6.4	40.9	59.1
Cys	11.6	69.0	31.1	11.4	0.2	99.8	6.4	44.7	55.3
Glu	2.5	67.5	32.5	6.6	9.9	90.1	4.1	37.6	62.4
Gly	5.3	68.5	31.5	18.0	14.1	85.9	9.2	40.5	59.5
Pro	4.1	71.8	28.2	8.6	13.3	86.7	4.4	45.0	55.0
Ser	4.2	78.4	21.6	11.7	14.7	85.3	6.3	44.5	55.5

1. Samples were assayed in 6 replicate cages (each with 6 birds).

2. Total CV = $\frac{\sqrt{\sigma e^2 + \sigma s^2}}{\bar{y}} \times 100\%$, where σs^2 = variance due to samples; σ_e^2 = variance due to cages within samples and $\bar{y}$ = respective mean of the PPI or wcDDGS or corn samples as shown in Table 4.3.

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.

variance components of the CV, it was observed that both for wcDDGS and corn, within sample variation (assay condition) was more than between sample variation (feed differences), whereas the reverse was true for PPI. Because DDGS has high fibre content (Nyachoti et al., 2005; Stein et al., 2006), which can cause differences in intake levels and consequently digestibility of nutrients among birds consuming the same sample, the within sample variation was more than the between sample variation in wcDDGS. Similar results have been reported previously by our lab (Bandegan et al., 2009). The variability in starch, protein, fibre and AA content of corn (Song et al., 2004) could cause variable feed intake levels of birds and therefore, greater within sample variation than between sample variation. The presence of ANF in peas is considered a great contributor to variability observed in the nutritive value of various samples. Also, because PPI is devoid of ANF and highly digestible, the total variation associated with the AID and the SID of each AA in PPI was observed to be greater between samples than within samples.

The AID and SID coefficients (Table 4.6 and 4.7) in a sample of wheat (AC Barrie) used as a reference were determined in each of the three independent experiments to assess the repeatability of the broiler ileal digestibility assay. The total inter- assay CV ranged from glutamic acid and proline to threonine and asparctic acid for AID (0.80% to 5.2%) and from proline to threonine for SID (0.6% to 4.5%). No significant differences ($P < 0.05$) were detected in the AID and SID of CP and AA among experiments. The variance components showed higher variation from within assays rather than between assays. These findings prove the reproducibility of the ileal digestibility assay used in our

Table 4.6. Apparent ileal CP and AA digestibility coefficients and their associated coefficient of variation (CV) determined in broiler chicks fed diets containing a reference wheat sample in 3 different assays[1]

Items	Assay					Variance components[3]		
	1	2	3	Average	CV,[2] %	Between assays (σs^2)	Within assays (σe^2)	*P*
CP	85.0	84.0	86.6	85.2	3.2	28.1	71.9	0.09
Indispensable AA								
Arg	81.0	79.4	81.9	80.8	3.8	3.2	96.8	0.34
His	85.4	84.2	84.8	84.8	3.2	0.0	100.0	0.67
Ile	85.0	85.7	87.2	86.0	3.2	10.6	89.4	0.24
Leu	86.3	86.4	87.8	86.9	2.3	7.9	92.2	0.28
Lys	77.2	76.2	78.6	77.3	3.9	2.2	97.8	0.36
Met	90.5	89.1	90.1	89.9	1.9	9.1	90.9	0.26
Met+Cys	88.4	87.6	88.6	88.2	1.7	0.0	100.0	0.45
Phe	87.6	87.3	88.4	87.8	2.4	0.0	100.0	0.56
Thr	73.4	74.1	77.0	74.8	5.2	31.8	68.3	0.07
Val	81.6	82.1	83.7	82.5	3.1	13.5	86.5	0.21
Dispensable AA								
Ala	78.6	77.3	80.2	78.7	4.1	18.2	81.8	0.16
Asp	76.5	75.6	77.1	76.4	5.2	0.0	100.0	0.40
Cys	85.6	86.4	87.3	86.4	3.1	6.8	93.2	0.29
Glu	94.9	94.5	95.1	94.8	0.8	16.3	83.7	0.18
Gly	80.0	79.0	81.0	80.0	3.0	3.4	96.6	0.34
Pro	93.5	93.1	93.6	93.4	0.8	0.0	100.0	0.46
Ser	82.7	83.1	84.8	83.5	3.0	21.4	78.6	0.14

1. Same sample of wheat was fed to 6 cages per assay (each with 6 birds).

2. Total CV = $\frac{\sqrt{\sigma e^2 + \sigma s^2}}{\bar{\gamma}} \times 100\%$, where σs^2 = variance due to samples; σ_e^2 = variance due to cages within samples and $\bar{\gamma}$ = average.

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 4.7. Standardized ileal CP and AA digestibility coefficients and their associated coefficient of variation (CV) determined in broiler chicks fed diets containing a reference wheat sample in 3 different assays[1]

Items	Assay					Variance components[3]		
	1	2	3	Average	CV,[2] %	Between assays (σs^2)	Within assays (σe^2)	*P*
CP	87.7	86.7	86.6	87.8	2.9	20.2	79.8	0.15
Indispensable AA								
Arg	83.8	82.0	83.8	83.1	2.7	0.00	100	0.40
His	87.8	86.4	87.1	87.1	3.0	0.00	100	0.61
Ile	88.6	89.0	90.6	89.4	3.0	8.63	91.4	0.27
Leu	89.2	89.0	90.4	89.5	2.2	6.64	93.4	0.29
Lys	81.1	80.0	82.5	81.2	3.8	2.77	97.2	0.35
Met	92.4	91.1	92.2	91.9	1.9	10.4	89.7	0.25
Met+Cys	91.4	90.4	91.5	91.1	1.5	1.45	98.6	0.37
Phe	92.8	92.5	93.7	93.1	2.3	0.34	99.7	0.55
Thr	82.8	82.9	85.8	83.8	4.4	25.5	74.5	0.11
Val	85.4	85.7	87.4	86.2	2.9	11.5	88.5	0.23
Dispensable AA								
Ala	82.5	81.0	83.9	82.5	3.9	17.4	82.6	0.17
Asp	81.8	80.7	83.4	82.0	4.5	0.00	100.0	0.45
Cys	89.4	89.9	91.0	90.1	2.2	5.09	94.9	0.32
Glu	95.9	95.4	96.1	95.8	0.8	16.9	83.1	0.18
Gly	83.7	82.5	84.6	83.6	3.0	4.47	95.5	0.33
Pro	95.2	94.8	95.3	95.1	0.6	0.69	99.3	0.38
Ser	87.3	87.6	89.3	88.1	2.8	18.5	81.5	0.16

1. Same sample of wheat was fed to 6 cages per assay (each with 6 birds).

2. Total CV = $\frac{\sqrt{\sigma e^2 + \sigma s^2}}{\bar{\gamma}} \times 100\%$, where σs^2 = variance due to samples; σ_e^2 = variance due to cages within samples; $\bar{\gamma}$ = average.

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.

laboratory and confirm that the digestibility values obtained in different experiments are comparable. Similar conclusions were also drawn previously in our lab (Bandegan et al., 2009).

4.6 CONCLUSION

Results of the present study show that overall AID and SID coefficients for PPI were 89.5% and 90.6%, for wcDDGS were 65.1% and 68.9%, and for corn were 82.1% and 88.4%, respectively. The SID estimates of indispensable AA in PPI indicated that lysine (95.7%), arginine (95.5%) and phenylalanine (96.1%) are the most digestible AA and SAA (87.2%) are the least. The SID estimates of indispensable AA in wcDDGS were evident with leucine (81.1%), and methionine (80.9%) as the most digestible, whereas, lysine (53.6%) and threonine (64.3%) as the least digestible. Among the indispensable AA in corn, SID estimates were highest for arginine (98.7%) and methionine (97.6%) and lowest for threonine (84.9%). The CV for AID and SID estimates in PPI, wcDDGS and corn were marked with lysine, leucine and methionine as the least variable, and SAA, lysine and threonine as the most variable indispensable AA. While, the CV for protein and AA contents were highest in corn, the CV for protein and AA digestibility estimates were highest in wcDDGS.

The digestibility dataset created in the current study for PPI, wcDDGS and Manitoba-grown corn would help nutritionists in including these feed ingredients in poultry diets since diets formulated on digestibility basis closely matches bird's AA and protein requirements, produces consistent performance, have narrow margins of safety, are cost-effective and minimizes nitrogen excretion.

5.0 MANUSCRIPT II

REQUIREMENT ESTIMATES OF DIGESTIBLE LYSINE AND DIGESTIBLE SULFUR AMINO ACIDS IN THREE-WEEK OLD BROILERS.

5.1 ABSTRACT

Two separate experiments (Exp.) were conducted to estimate the requirements of dietary digestible lysine (dLYS) (Exp. 1) and of dietary digestible SAA (dSAA) (Exp. 2) in three-week old broilers. One hundred eighty (Exp. 1) or 150 (Exp. 2) Ross 308 male broilers were fed wheat-DESBM-based diets deficient in lysine (Exp.1) or SAA (Exp.2). Six graded levels of dietary dLYS (Exp. 1) varying from 0.80% to 1.30% and five graded levels of dietary dSAA (Exp. 2) varying from 0.52% to 0.92% were assayed. The experimental diets were kept isocaloric and isonitrogenous by incorporation of dextrose or vegetable oil and glutamic acid, respectively. On day 0, birds were weighed and randomly distributed to either 36 (Exp.1) or 30 cages (Exp.2) and allowed a*d-libitum* access to experimental diets and water throughout the experimental period. Body weight and feed intake per cage were recorded on day 0 and day 21 to calculate body weight gain (BWG) and feed conversion ratio (FCR). At the end of the experiment, all birds were sacrificed by cervical dislocation and skin, feathers, head, toes and viscera were removed. Skinless whole carcass (rack + breast meat + thigh + drumstick), breast muscle and thigh + drumstick were weighed separately to calculate yield (as percentage of live

bird weight) of carcass (CY), breast muscle (BMY) and thigh + drumstick (TDY), respectively. The dietary dLYS and dietary dSAA requirement estimates obtained from significant quadratic ($P < 0.05$) regression equations were 1.12% and 0.81% for optimum BWG, respectively. The obtained dietary dLYS requirement estimates for optimum FCR was 1.13%, while no significant response was observed for FCR to dSAA levels. The CY and BMY increased cubically with increasing levels of dietary dLYS, whereas, CY showed no significant response. The BMY increased linearly with increasing levels of dietary dSAA. No effect was observed on TDY in either experiment.

5.2. INTRODUCTION

Methionine and lysine are the first and the second most limiting AA in practical broiler diets and, therefore, research in the area of broiler AA requirements has mostly been centered on them. The NRC (1994) recommendations for lysine and methionine are considered inadequate for optimum production of modern broilers (Han and Baker, 1991, 1993; Baker and Han, 1994; Kidd et al., 1997). Firstly, because NRC (1994) recommendations are derived from broiler studies conducted two decades ago and, secondly, because these recommendations (NRC, 1994) present total rather than digestible AA requirements. Considering that the AA availability of a feed ingredient is better judged by its digestible AA content rather than the total AA content (Ravindran and Bryden, 1999), it is more accurate to describe the AA requirements of a bird in terms of digestible rather than total values. Birds fed digestible AA based diets perform better than those fed total AA based diets (Dari et al., 2005). Therefore, in order to best utilize

the genetic potential of modern broilers, several studies have been conducted to establish/ re-establish the digestible AA requirement. The requirements of a bird for AA vary with genetics, age, sex, diet and environment, and, therefore, it is not practical to determine the requirements for each AA under every practical condition. The poultry researchers at University of Illinois established “ideal chick protein ratios” (also known as Illinois ideal chick protein concept, IICP) using digestibility coefficients (Han and Baker, 1994; Baker, 1997). In this concept, lysine was considered as a reference AA and levels of all other AA were determined in proportion to lysine. However, it was assumed that though absolute requirements of AA may differ, established AA to lysine ratios would remain same under all practical conditions (Han and Baker, 1994; Baker, 1997). Therefore, optimum requirements have to be determined only for lysine under different production conditions, and optimum requirements for all other AA are obtained using optimum ratios (Baker, 1997).

Body weight gain and feed conversion ratio are the usual response criteria whenever requirements are estimated, but profitable broiler production could be better expressed in terms of carcass attributes. This is because the market for breast meat is increasing, and the optimum AA requirements for CY are different from those for growth performance. For instance, the requirements for lysine have been found to be higher for optimum BMY than for optimum growth (Jensen et al., 1989; Acar et al., 1991; Bilgili et al., 1992; Holsheimer and Ruesink, 1993; Han and Baker, 1994; Renden et al., 1994; Kidd et al., 1998; Kerr et al., 1999; Corzo et al., 2006). Hence, both growth performance and processing yield should be considered when AA requirements are to be derived.

Breast muscle constitutes 30% of total edible meat in the carcass and 75% of the breast meat is protein (Summer and Leeson, 1985). Respectively, lysine and methionine represent the highest and the lowest content among AA of breast meat (Kerr et al., 1999). It was observed that dietary lysine increases breast muscle accretion (Sibbald and Wolynetz, 1986; Renden et al., 1986; Moran and Bilgili, 1990; Gormon and Balnave, 1995; Labadan et al., 2001) but broilers fed diets containing high lysine require additional supplementation of methionine to optimize BMY (Hickling et al., 1990; Schutte and Pack, 1995a; Huyghebaert and Pack, 1996). Moreover, it has also been reported that lysine levels in starter diets influences the BMY in grower-finisher broilers (Holsheimer and Ruesink, 1993; Kerr et al., 1999; Kidd et al., 1998). Considering the above findings, it would be of interest to study the effect of graded levels of methionine and lysine on CY and BMY at 21-day of age and also to determine if requirements of lysine and SAA are higher for maximum BMY than for maximum BWG. Since TDY has been found to be inversely related to BMY (Kerr et al., 1999), the requirements of lysine and SAA in relation to TDY should also be studied.

5.3. OBJECTIVES

Two separate experiments were conducted with the objective to derive the requirements of dLYS and dSAA for optimum growth performance (BWG and FCR) and optimum processing yield (CY, BMY and TDY) in three-week old broilers, when all AA, except those under test are in proportion according to IICP (Baker and Han, 1994).

5.4. MATERIALS AND METHODS

5.4.1 DIETS

Dry extruded-expelled soybean meal (DESBM) (Jordan Mills Inc., Delmar Commidities Ltd., Winkler, Manitoba, Canada) and wheat (James farm, Winnipeg, Manitoba, Canada)-based diets deficient in lysine (Exp. 1) or SAA (Exp. 2) were formulated. The total AA composition of DESBM and wheat (Table 5.1) were analyzed using ion-exchange chromatography with post-column derivatization with ninhydrin (Llames and Fontaine, 1994). Sulfur AA were first oxidized with performic acid, and subsequently neutralized with sodium metabisulfite for analysis (Commission Directive, 1998). The AA concentrations were quantified with the internal standard method by measuring the absorption of reaction products with ninhydrin at 570 nm.

The digestible AA values in DESBM and wheat (Table 5.1) were calculated from SID coefficients (estimated previously in our lab by Bandegan, 2010) and the total AA contents analyzed. The calculated digestible levels of AA were used in formulation of basal lysine (Exp. 1) or SAA (Exp. 2)-deficient diets to meet requirements of all indispensable AA, except those under test, according to the IICP for 3-week old broilers. The requirements of all other nutrients either met or exceeded NRC (1994)

Table 5.1. The SID[1] coefficients, analyzed total AA and calculated[2] digestible AA in wheat and DESBM

Item	SID coefficients		Total AA values		Calculated digestible values	
Amino acid (%)	Wheat	DESBM	Wheat	DESBM	Wheat	DESBM
Arginine	85.2	92.9	0.53	2.41	0.45	2.24
Histidine	87.1	89.6	0.30	0.93	0.26	0.84
Isoleucine	90.4	89.5	0.43	1.48	0.39	1.33
Leucine	90.5	89.6	0.86	2.57	0.78	2.30
Lysine	83.7	90.8	0.41	2.26	0.34	2.05
Methionine	91.4	92.5	0.19	0.41	0.17	0.38
Met+Cys[3]	91.1	87.5	0.45	0.98	0.41	0.86
Phenylalanine	93.8	95.9	0.60	1.76	0.57	1.69
Threonine	85.4	88.0	0.38	1.44	0.33	1.27
Valine	87.7	88.4	0.51	1.62	0.44	1.43
Alanine	83.8	89.0	0.48	1.55	0.40	1.38
Aspartic acid	83.8	87.7	0.70	3.91	0.58	3.43
Cystine	90.8	82.5	0.26	0.57	0.24	0.47
Glutamic acid	90.6	91.4	3.73	6.44	3.60	5.89
Glycine	84.1	86.0	0.54	1.54	0.45	1.32
Proline	95.4	89.4	1.18	1.85	1.12	1.65
Serine	89.1	88.6	0.61	1.84	0.54	1.63

[1]SID coefficients adapted from Bandegan et al. (2010).
[2]Digestible levels of AA in wheat and dry extruded expelled soybean meal (DESBM) were calculated from respective SID coefficients and analyzed total AA values.
[3]Sulphur AA.

recommendations. The desired graded levels of L-Lysine or DL-Methionine and L-Cysteine were added to the basal diets (Table 5.2 and Table 5.3) at the expense of glutamic acid and dextrose to formulate six (Exp. 1) or five (Exp. 2) dose-response diets, respectively. The dietary digestible lysine levels assayed (Exp. 1) were 0.80%, 0.90%, 1.0%, 1.10%, 1.20% and 1.30% and corresponding calculated total lysine levels were 0.91%, 1.01%, 1.11%, 1.21%, 1.31% and 1.41% (at 87.1% digestibility). The dietary dSAA levels (Exp. 2) assayed were 0.52%, 0.62%, 0.72%, 0.82% and 0.92% and corresponding calculated total SAA levels were 0.59%, 0.69%, 0.79%, 0.89%, and 0.99% (at 89.9% digestibility). All experimental diets were kept isonitrogenous and isocaloric and were balanced for vitamins and minerals.

5.4.2 BIRDS AND CONDUCT OF EXPERIMENT

The experimental protocol 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).

One-day old 180 (Exp.1) or 150 (Exp.2) Ross 308 male broiler chicks were obtained from a local hatchery (Carlton, Grunthal, Manitoba, Canada) and were housed for 21-days in Petersime battery brooders (Petersime Incubator Co., Gettysburg, Ohio) placed in well-illuminated (24 h) fluorescent lighting rooms. The initial temperature of the room was kept at 32°C and was reduced weekly by 4°C. The chicks were weighed

Table 5.2. Ingredients of lysine (Exp. 1) and sulfur (Exp. 2)-deficient basal diets, as fed basis [1]

Ingredient %	Experiment 1	Experiment 2
HRS Wheat	40.54	53.0
Ext. SBM	32.5	31.4
Vegetable oil	3.3	7.0
Dextrose	10.55	0.697
Cellulose	5.97	-
Glutamine	1.79	2.80
Limestone	1.39	1.44
Biophos	1.65	1.55
Vitamin/mineral premix[2]	1.5	1.5
L-Arginine	0.14	0.11
L-Lysine.HCl	-	0.23
L-Valine	0.13	0.09
L-Isoleucine	0.08	0.05
L-Threonine	0.12	0.10
L-Leucine	0.06	-
L-Cysteine	0.11	-
DL-Methionine	0.17	0.033

[1]Diet formulation is based on digestible AA in wheat and dry-extruded expelled soybean meal calculated as explained in Table 5.1.

[2]Vitamin and mineral premix included the following per kilogram of diet: vitamin A, 1 million IU/g; vitamin D_3, 500,000 IU/g; vitamin E, 100,000 IU/g; riboflavin, 7921mg; pantothenic acid, 14507 mg; niacin, 22088 mg; choline, 290116 mg; vitamin B_{12}, 1000 mg/kg; folic acid, 96% ; thiamine, 81%; biotin, 20000 mg/kg; virginiamycin (Stafac-22), 22 g/kg; monensin sodium (Coban), 200 g/kg; MnO, 60%; ZnO, 72% ; $CuSO_4$, 25.2% ; $FeSO_4$, 31% ; calcium iodate premix, 8.9% ; sodium selenite, 0.2%.

Table 5.3. Calculated composition of digestible lysine- and digestible SAA -deficient diets used in Exp. 1 and Exp. 2, respectively

Calculated composition	Experiment 1	Experiment 2
ME, kcal/kg	2624	2921
Crude protein, %	20.7	22.9
Digestible SAA, %	0.72	0.52
Digestible Met, %	0.36	0.28
Digestible Cys, %	0.36	0.28
Digestible Lys, %	0.80	1.00
Digestible Arg, %	1.05	1.05
Digestible His, %	0.38	0.40
Digestible Phe, %	0.78	0.83
Digestible Thr, %	0.67	0.67
Digestible Leu, %	1.12	1.13
Digestible Ile, %	0.67	0.67
Digestible Val, %	0.77	0.77
Calcium, %	0.94	0.94
Available Phosphorus, %	0.45	0.45

on day 1 and randomly assigned to cages such that each cage had similar initial weight. In each experiment six cages of five birds each were assigned to each of six (Exp. 1) or five (Exp. 2) experimental diets in a completely randomised design. The experimental diets and water were offered for *ad-libitum* consumption.

Body weight and feed intake were recorded at the beginning and the end of the experiment to calculate BWG and FCR over the 21-day experimental period. Weight of dead birds was taken into account to correct for calculated average BWG and FCR. Also, at the end of the experiments, birds were sacrificed by cervical dislocation and skin, feathers, head, feet, and viscera were removed. Skinless carcass (breast muscle + rack + thigh + drumstick), breast meat (Pectoralis major and Pectoralis minor) and thigh + drumstick were weighed to calculate CY, BMY and TDY.

5.4.3 STATISTICAL ANALYSIS

Growth performance and processing yield data from the completely randomised design was evaluated using GLM procedure of SAS (SAS, Institute Inc, 1990) and treatment means were compared using Tukey's test when treatment effects were found to be significant ($P < 0.05$). These data were also subjected to Proc Reg (Draper and Smith, 1981) to determine the best fit regression between dependent(y) and the independent (x) variables using the following polynomial model: $y_{ij} = b_o + b_1x_i + b_2x_i^2 + b_1xi^3 + e_{ij,}$ where $y_{ij} =$ response criteria measurement on j'th bird in i'th level of dLys or dSAA, x_i = dLys or dSAA level, bo = intercept, b_1 = regression on x_i, b_2 = regression on x_i^2, b_3 = regression on x_i^3 and $e_{ij} =$ error deviation. The highest significant polynomial terms, among linear,

quadratic and cubic terms was determined and used for interpretation of data. Equations found to have significant ($P < 0.05$) quadratic terms (and not significant cubic terms) were used in subsequent analysis. This was done by setting the first derivative of the quadratic equation to zero, and solving the resultant equation and checking for the maximum or minimum response.

5.5. RESULTS AND DISCUSSION

5.5.1 EXPERIMENT 1

The calculated and analyzed contents of total lysine in respective experimental diets were in close agreement (Table 5.4). Therefore, the requirement estimates obtained using calculated dLYS levels (Table 5.6) were very similar to those obtained using analyzed dLYS levels (Appendix 9.2). A significant quadratic response ($P < 0.01$) was observed for BWG and FCR with increased levels of digestible lysine (Table 5.5). Using quadratic regression equations, the estimated dietary dLYS required to obtain maximum BWG (1.12%) was very close to dLYS levels required to obtain maximum FCR (1.13%) (Table 5.6). Corresponding total lysine values (at 87.1% digestibility) were 1.29% and 1.30%. Our results are in agreement with the findings of Kidd et al. (1997) that starter chicks fed diets containing 1.20% total lysine have improved BWG and FCR than those fed diets containing 1.10% total lysine.

A number of studies have been conducted to estimate lysine requirements in starter chicks, but results obtained from these studies are quite variable. These variations have been attributed to genetics (Bilgili et al., 1992), response criteria (Sibbald and Wolynetz,

Table 5.4. Calculated and analyzed total lysine (Exp. 1) and total sulfur AA (Exp. 2) content in experimental diets

Item		Experimental diets					
		Experiment 1					
Total AA (%)		1	2	3	4	5	6
Lysine	Calculated	0.91	1.01	1.11	1.21	1.31	1.41
	Analyzed	0.92	1.00	1.15	1.23	1.28	1.41
		Experiment 2					
		1	2	3	4	5	
Met+Cys	Calculated	0.59	0.69	0.79	0.89	0.99	
	Analyzed	0.81[1]	0.79	0.83	0.93	1.09	

[1]Analyzed values greater than 0.1 of calculated values were considered different, therefore experimental diet containing 0.52% dietary dSAA (or 0.59% SAA, based on 89.9% digestibility) was eliminated from statistical analysis of data.

Table 5.5. Growth and yield performance of broilers[1] fed graded levels of digestible lysine from day 0 to 21 (Exp. 1)

Digestible Lysine (% of diet)	Growth performance parameters		Processing yield parameters (%, of live wt.)		
	BWG[2] (g/bird)	FCR[2] (g/g)	CY[2]	BMY[2]	TDY[2]
0.80	315	3.02	47.5	6.96	19.1
0.90	434	2.58	51.3	10.2	19.3
1.00	578	2.20	52.5	13.5	18.9
1.10	673	1.97	53.6	15.3	18.6
1.20	567	2.23	51.5	12.2	18.8
1.30	527	2.30	53.2	14.3	19.0
SEM[3]	19.2	0.05	0.62	0.28	0.25
Probability----------					
Linear	0.0001	0.0001	0.0029	0.0064	NS
Quadratic	0.0001	0.0001	0.0048	0.0152	NS
Cubic	NS[4]	NS	0.0073	0.0297	NS

[1]Values are means of 6 replicates with 5 birds per pen.
[2]BWG= body weight gain, FCR = feed conversion ratio, CY= Carcass yield, BMY= breast muscle yield, TDY= thigh + drumstick yield.
[3]Standard error mean.
[4]NS = not significant.

Table 5.6. Estimated requirements based on quadratic regression analysis of digestible lysine (%) and digestible SAA (%) in 3-week old broilers

Amino acid (% of diet)	Response criteria	Equation[1]	R^2	Requirement estimates
Digestible Lysine	BW gain, g	-3337.9 + 7070.1 × (lysine) - 3153.9 × (lysine × lysine)	0.82	1.12
	FCR, g/g	13.588 – 20.459 × (lysine) + 9.0804 × (lysine × lysine)	0.86	1.13
Digestible SAA	BW gain, g	-1191.1 + 5029 × (SAA) - 3116.9 × (SAA × SAA)	0.45	0.81

[1]Prediction equation based on formulated digestible lysine or digestible SAA for optimum response

1986, 1987; Rhodehutschord and Pack, 1999; Baker et al., 2002; Lumpkins et al., 2007), ingredient quality, nutrient levels and environmental factors (Kidd and Fancher, 2001).In addition, the mathematical model or statistical approach used for estimation could also lead to considerable differences in recommendations (Leclerq, 1998; Rhodehutschord and Pack, 1999; Baker et al., 2002). For instance, Baker et al. (2002) reported 1.08% and 1.15% as the lysine requirement for maximum BWG and FCR, respectively, based on quadratic regression equations. Whereas, corresponding values based on the broken line regression method were 0.85% and 0.97%. Similarly, Dozier et al. (2009) reported 1.07% and 1.10% as subjective (95% of maximum response) estimates for optimum BWG and FCR using the quadratic regression analysis, but obtained 1.09% and 1.15% using the broken-line quadratic model. Hence, it is difficult to compare requirement estimates obtained from different studies. While, the regression analysis has been considered as a better method to describe dose-related response to nutrients than the broken line model which underestimates the amount of nutrients required for optimum performance (Mack et al., 1999), the broken line methodology has been used widely in requirement studies with an arbitrary point set either at 90% (Baker and Han, 1994; Baker et al, 2002) or 95% (Dozier et al., 2009) of maximum response. In contrast, estimates reported in the present study were not based on any subjective estimation. If, the present data were also estimated subjectively (90% or 95% of maximum response) the dietary dLYS levels required for maximum BWG and FCR become 1.01% to 1.03% and 1.06 to 1.08%, respectively. Interestingly, these subjective estimates concur well with previous estimates reported for optimum BWG (Han and Baker, 1991, 1993; Vazquez and Pesti, 1997; Knowles and Southern, 1998; Labadan et al., 2001; Zaghari et al., 2002; Garcia and Batal, 2005) and for

optimum FCR (Vazquez and Pesti, 1997; Knowles and Southern, 1998; Garcia and Batal, 2005; Dozier et al., 2009). Therefore, comparisons among dose-response studies should be made with caution.

The CY and BMY showed a cubic response ($P < 0.01$ and $P < 0.03$) with increased levels of dietary dLYS (Table 5. 6). Cubic increases in breast weight and proportional BMY at increasing dietary lysine levels have been reported previously in grower-finisher broilers (Hickling et al., 1990 in 6 week old Ross × Arbor Acres; Acar et al., 1991 in 6 to 8 week and Han and Baker, 1994 in 3 to 6 week old Ross × Ross; Kerr et al., 1999 in 7 week old Peterson × Arbor Acres male broilers). To the best of our knowledge, no such study conducted in starters is available for comparison purposes. The increased lysine (1.30% of diet) in starter diets has been shown to increase the deposition of protein in 2-week old broilers (Holsheimer and Ruesink, 1993). It has also been reported that lysine required for maximum protein accretion is more than that required for maximum body weight (Sibbald and Wolynetz, 1986; Holsheimer and Ruesink, 1993) as well as higher than that considered adequate for feed efficiency (Hickling et al., 1990; Moran and Bilgili, 1990; Acar et al., 1991; Bilgili et al., 1992; Renden et al., 1994; Gorman and Balnave, 1995; Kidd et al., 1998). Interestingly, increases in CY and BMY from 1.20% to 1.30% dietary dLYS levels were not accompanied by increase in BWG. Increased muscle mass without corresponding increase in body weight could be due to decreased abdominal or muscle fat content, as suggested by others (Hickling et al., 1990; Renden et al., 1984; Han and Baker, 1991; Kerr et al., 1999). The percentage of CY and BMY was highest in birds fed dLYS at 1.10 % of diet. This was also the level of lysine at which optimum weight gain and feed efficiency were obtained. Therefore, the dLYS required for maximum CY and BMY are

either equal to or higher than the levels required to obtain maximum BWG and FCR. The requirements for optimum CY and BMY could have been better derived if higher graded levels (higher than 1.30%) of dietary dLYS were assayed. No significant response was observed in TDY with increased levels of dietary dLYS. This current finding in starters was in agreement (Holsheimer and Ruesink, 1993) or in disagreement (Kerr et al., 1999) with previous findings in grower-finishers.

It was observed that the levels of dLYS required to optimize BWG is similar to those required to maximize FCR. Similar trends have been reported for lysine (Labadan et al., 2001; Garcia et al., 2006), and SAA (Mendonca and Jensen, 1989; Morris et al., 1992; Huyghebaert and Pack, 1996; Aftab and Ashraf, 2009) as well as for tryptophan, threonine, isoleucine and valine (Baker et al., 2002). In contrast, higher requirements for optimum FCR than BWG for lysine (Han and Baker, 1993; Baker and Han, 1994; Garcia and Batal, 2005; Baker et al., 2002; Zaghari et al., 2002) and for SAA (Han and Baker, 1993, 1994; Baker et al., 2002; Mack et al., 1999; Lumpkins et al., 2007) have also been reported.

5.5.2 EXPERIMENT 2

Except for the diet formulated to contain 0.52% dSAA, the calculated and analyzed SAA levels in other dose-response diets were in close agreement (Table 5.4). Therefore, data from only 4 experimental diets (diets containing dSAA levels 0.62%, 0.72%, 0.82% and 0.92%) were used in the statistical analysis. The BWG responded in a quadratic

Table 5.7. Growth and yield performance of broilers[1] fed graded levels of digestible SAA from day 0 to 21 (Exp. 2)

Digestible SAA (% of diet)	Growth performance parameters		Processing yield parameters (%, of live wt.)		
	BWG[2] (g/bird)	FCR[2] (g/g)	CY[2]	BMY[2]	TDY[2]
0.62	729	1.25	52.8	13.3	18.8
0.72	815	1.26	53.7	14.8	18.8
0.82	836	1.21	53.2	14.3	18.5
0.92	798	1.27	53.8	15.1	18.5
SEM[3]	20.1	0.015	0.56	0.29	0.24
Probability----------					
Linear	0.003	NS	NS	0.002	NS
Quadratic	0.005	NS	NS	NS	NS
Cubic	NS[4]	NS	NS	NS	NS

[1]Values are means of 6 pens each with 5 birds.
[2]BWG= body weight gain, FCR = feed conversion ratio, CY= Carcass yield, BMY= breast muscle yield, TDY= thigh+drumstick yield.
[3]Standard error mean.
[4]NS = not significant.

manner ($P < 0.05$) with increasing levels of dietary dSAA from 0.62 to 0.92% (Table 5.7). The requirement estimates obtained from calculated dSAA (Table 5.6) and analyzed dSAA values (Appendix 9.2) are closely related. In the current study the analyzed values within 10% of calculated values were considered similar. Using quadratic regression analysis the dietary dSAA estimate for BWG was 0.81% (Table 5.6). The corresponding total SAA levels (at 89.9% digestibility) were 0.90%. No significant response was observed for FCR with increasing dSAA levels. The dSAA estimates obtained for BWG were in agreement with some studies (Baker and Han, 1994 - 0.78%; Garcia and Batal, 2005- 0.75% or 0.83%; Dozier et al., 2009 - 0.85%) and were in disagreement with others (Knowles and Southern, 1998 obtained a requirement of 0.66 at 1.00% dLYS; Aftab and Ashraf, 2009 reported 0.67%).

Our dSAA estimates of 0.81% for 21-days are also similar to a dSAA estimate of 0.80% for 7-day old broilers (Sklan and Noy, 2003). While some researchers have considered NRC (1994) SAA recommendations to be higher than required (Knowles and Southern, 1998; Aftab and Ashraf, 2009), our results suggest NRC (1994) recommendations for total SAA requirement (0.90%) in 21 day old broilers to be adequate.

Due to a lack of significant quadratic response for CY, BMY and TDY to increasing levels of dietary dSAA, no requirements estimates for these response criteria was determined. The BMY increased linearly with increasing levels of dietary dSAA, indicating that optimum BMY was not obtained from the graded levels of dSAA used in this experiment; therefore, the level of dietary dSAA required for maximum BMY might be higher than 0.92%. No published report was available for comparison of yield

performance to graded levels of SAA at 21 day of age in broilers. Again, as stated previously that differences in feed ingredients used as well as response criteria, mathematical approach and statistical analysis could lead to variations in AA requirements estimated by different researchers, therefore, comparison between studies should be made with caution.

Though it was not the aim of the present study to predict the requirements of SAA from lysine requirements derived in Exp.1 and compare them with SAA requirements derived in Exp. 2, the use of similar diets (feed ingredients, feed formulation based on digestible ideal ratios), birds (strain, age) and environmental conditions, as well as the use of similar statistical analysis in Exp 1 and Exp. 2, provided us an opportunity to do so. The lysine requirements obtained in Exp. 1 were 1.12% and 1.13% for maximum BWG and FCR , and 0.72 (ideal SAA:lysine suggested by Baker and Han, 1994) of these levels are approximately 0.80% and 0.81% , which are very similar to the dSAA requirements obtained in Exp. 2. Using the average of requirements (obtained in the present study) optimum dietary ratio of dSAA to dLYS was calculated to be 0.715:1 which is similar to IICP recommendation of 0.72:1 for 0 to 21 day old broilers (Baker and Han, 1994).

5.6. CONCLUSION

The dLYS levels of 1.12% and 1.13% and dSAA levels of 0.81% were estimated as requirements for maximum BWG and maximum FCR, respectively. It can be concluded that the AA levels (lysine or SAA) required to optimize BWG did not differ from the

levels required to maximize FCR, whereas the lysine required to obtain maximum CY and BMY might be higher than levels required to optimize growth performance. The estimates obtained in current study consider NRC (1994) recommendations of lysine and SAA for three-week old broilers to be adequate. Though not addressed in this study, but economics of input and output variables should be considered before using the requirement recommendations from dose response studies in practical feed formulations.

6.0 GENERAL DISCUSSION

The first objective of the current study was to determine the SID of protein and AA in PPI, wcDDGS and corn in 3-week old broilers. The ileal protein and AA digestibility in broilers is a reliable estimate of AA availability in a given feed ingredient (Stein et al., 2007a). However, due to variable amounts of IEAL, the AID coefficients of AA vary with the dietary protein intake levels (Fan et al., 1994). The SID coefficients derived by correcting AID values for basal IEAL have been considered to be a more accurate representative of AA availability (Lemme et al., 2004). Our previous studies have reported that the differences in methodology and inherent properties of feed ingredients could cause differences in digestibility estimates both within and between feed samples (Bandegan et al., 2009b, 2010). This can call into question the accuracy of diet formulation, increases safety margins and thus cost of feed. Therefore, the CV associated with digestibility estimates of AA in feed ingredients was also determined.

The AA contents in feed ingredients have been related to AA composition of storage protein. For example, the storage protein in peas is high in lysine and low in SAA (Croy et al., 1980), while the storage protein in cereals (wheat and corn) is low in lysine. Therefore, PPI had high content of lysine (5.6%) and low content of SAA (1.6%), whereas, both wcDDGS and corn had low contents of methionine (0.75% and 0.21%) and lysine (0.82% and 0.23%) and high contents of leucine (3.65% and 1.12 %), respectively. The AA contents of corn were observed to be thrice the AA contents of wcDDGS. Similar reports have been obtained previously both in pigs and poultry

using wheat-DDGS (NRC, 1994; Nyachoti et al., 2005; Thacker and Widyaratne, 2007; Lan et al., 2008; Bandegan et al., 2009b).The methionine was least and isoleucine and leucine were most variable indispensable AA in PPI. On the other hand, methionine was the most and valine was the least variable indispensable AA of wcDDGS and corn. Overall, the CV associated with AA composition was highest in corn followed by PPI and wcDDGS.

The overall average SID estimates of AA in PPI, wcDDGS and corn were 93.3%, 71.5% and 93.25%, respectively. The negligible contents of ANF and cell wall in PPI (Gatel and Grosjean, 1999; Le Guen et al., 1995) could be responsible for higher digestibility of PPI compared to peas. The high digestibility estimates of arginine and lysine in PPI could be due to high enzymatic hydrolysis of these AA (Low, 1980), and the low digestibility estimates of methionine and cysteine might be due to presence of protease inhibitors in peas which divert SAA from body tissue synthesis to additional production of pancreatic proteases. (Gatel, 1994; Wiseman et al., 2003). It was observed that AA digestibility estimates were related to the AA contents of feed ingredients. Therefore, the SID of methionine was the lowest (87.2%) and highest (97.6%, 80.9%) among indispensable AA of PPI, wcDDGS and corn, respectively. The lysine was found to be the most variable and least digestible AA in wcDDGS with AID and SID estimates of 50.7% and 53.6%, respectively. The lower availability of lysine in DDGS could be the result of heat damage of these AA (Batal and Dale, 2006; Fastinger et al., 2006). At high drying temperatures, maillard reactions occur and lysine gets attached to reducing sugars and becomes cell bound which protects them from digestive processes making them poorly digestible (Mauron, 1981).

Not many studies were available for comparing the digestibility data. The digestibility estimates obtained for PPI were higher than AID estimates of Valencia et al (2009). This could be due to different process used for PPI production. Since the AA composition of DDGS depends upon the composition of grains used and production technology it is apparent that the AA digestibility would vary. Also, different growing and cultivation conditions affect the nutrient composition of corn so does the AA digestibility. Moreover, digestibility estimates vary with genotype, age and type of bird, method of determination (Ten Doeschate et al., 1993; Huang et al., 2006) as well as protein content of the feed ingredient (Fan et al., 1994) therefore AA digestibility comparisons across different studies is difficult.

The CV associated with the SID estimates were the highest and widest (6.6% to 29.4%) in wcDDGS, followed by corn (3.2% to 10.7%), and PPI (2.7% to 13.5%) in that order. Along with threonine, SAA in PPI, and lysine in wcDDGS and in corn were the most variable and least digestible indispensable AA, whereas leucine and methionine were the least variable indispensable AA in all the three feed ingredients assayed in this study. In congruence to our previous study in broilers (Bandegan et al., 2009b) the within sample variations were observed to be more than between sample variations among wcDDGS samples. This could be explained by the fact that DDGS has a high fibre content (Nyachoti et al., 2005; Stein et al., 2006), which could cause differences in intake levels and consequently the differences in digestibility of AA among birds. Similarly, starch, fibre, protein and AA contents among corn samples can vary considerably (Coweison, 2005) leading to the variability in bird intake levels of nutrients, therefore the CV for within samples showed more variation than between samples. Whereas, the reverse was true for PPI and reason for this finding is not

known. It was observed during experiment that PPI being fine powder was not very palatable. Therefore, if mixture of PPI and wheat in 50:50 was used as an assay diet and AA and protein digestibility in PPI was calculated by difference method the partition of CV could have been better defined

The AID coefficients in a sample of wheat (AC Barrie) used as a reference were determined in a series of three independent experiments (Table 4.7) to assess repeatability of the broiler ileal digestibility assay. The total inter-assay CV ranged from 0.8% to 5.2% for glutamic acid and proline to threonine and aspartic acid, respectively. No significant differences ($P < 0.05$) were detected in the AID and SID of CP and AA among experiments. The variance components showed higher variation from within rather than between assays. These findings prove the reproducibility of the ileal digestibility assay used in our laboratory and confirm that the digestibility values obtained in different experiments are comparable.

The second objective of the current study was to determine the optimum requirement of dLYS and dSAA for maximum growth (BWG and FCR) and maximum processing yield performance (CY, BMY and TDY) in 3-week old broilers. The SAA and lysine are the most limiting AA in practical broiler diets; therefore required supplementation of SAA and lysine in broiler diets results in improved performance. It has been observed that AA requirements in broilers vary with external (environment, diet) and internal (genetic) factors but it is not possible to estimate AA requirements under all combination of conditions and genotype (Emmert and Baker, 1997). Therefore, a reference AA was chosen and all other indispensable AA were expressed in fixed proportion to the reference AA to yield ideal AA ratios (Han and Baker, 1994). The ideal AA ratios (also known as ideal protein concept) refer to the

levels of all the indispensable AA present in sufficient amounts i.e. without any excesses or deficiencies (Emmert and Baker, 1997). And such ratios are established using digestibility coefficients to overcome the differences in dietary AA absorption and utilization, which can cause variations in AA proportions (Baker, 1997).

The six graded levels of dietary dLYS (0.80%, 0.90%, 1.00%, 1.10%, 1.20% and 1.30%) and the five graded levels of dietary dSAA (0.52%, 0.62%, 0.72%, 0.82% and 0.92%) were assayed. The significant quadratic response ($P < 0.01$) was observed for BWG and FCR with increased levels of dietary dLYS (1.12% and 1.13%) and for BWG (0.81%) with increased levels of dietary dSAA. While no significant response was observed for FCR with increased levels of dietary dSAA. The requirement estimates to obtain maximum BWG and FCR were similar for dLYS. Similar trends have also been reported by others for lysine (Labadan et al., 2001; Garcia et al., 2006) as well as SAA (Mendonca and Jensen, 1989; Morris et al., 1992; Huyghebaert and Pack, 1996).

It has also been reported that lysine levels in starter diets influence the BMY in grower-finisher broilers (Holsheimer and Ruesink, 1993; Kidd et al., 1998; Kerr et al., 1990) but broilers fed diets containing high lysine require additional supplementation of methionine to optimize BMY (Hickling et al., 1990; Schutte and Pack, 1995a; Huyghebaert and Pack, 1996). The CY and BMY increased cubically ($P < 0.01$ and $P < 0.03$) with increased levels of dietary dLYS while BMY increased linearly with increased levels of dSAA in diet. Interestingly, increases in CY and BMY from 1.20% to 1.30% dietary dLYS levels were not accompanied by increase in BWG. However, numerical percentage of CY and BMY was highest in birds fed dLYS at 1.10 % of diet. This was also the level of lysine at which optimum weight

gain and feed efficiency were obtained. Therefore, levels of dLYS required to obtain maximum CY and BMY are either equal to or higher than the levels required to obtain maximum BWG and FCR. No studies in starters were available for direct comparison of results but similar findings have been reported in finishers by others (Sibbald and Wolynetz, 1986; Holsheimer and Ruesink, 1993; Bilgili et al., 1992; Gorman and Balnave, 1995). No significant response was observed in TDY with increasing levels of dietary dLYS and in CY and TDY with increasing levels of dietary dSAA.

The AA requirement estimates for broiler performance varies due to genetics (Bilgili et al., 1992), response criteria (Sibbald and Wolynetz, 1986, 1987; Rhodehutschord and Pack, 1999), mathematical model or statistical approach used (Leclerq, 1998; Rhodehutschord and Pack, 1999; Baker et al., 2002) ingredient quality, nutrient levels and environmental factors (Kidd and Fancher, 2001). Therefore comparisons between different AA requirement studies were difficult.

Since similar methodology (age and strain of bird), feed ingredients and environmental conditions were used in both experiments, SAA requirements were predicted from lysine requirement values using ideal AA ratios. The levels of SAA required for optimum performance were 72% of the levels of lysine required for optimum performance. The predicted requirement of SAA was very close to experimentally obtained requirement value. Also, these recommendation ratios were similar to IICP recommendations for Lys:SAA in 0 to 21 day old broilers (Baker and Han, 1994).

7.0 GENERAL CONCLUSIONS

The SID coefficients for CP in PPI, wcDDGS and corn were 90.6%, 68.9%, 88.4%. Methionine was the most digestible indispensable AA in wcDDGS and corn but was the least digestible indispensable AA in PPI. Threonine was the least digestible AA in all feed ingredients assayed. Lysine was among the least digestible AA in wcDDGS and corn, whereas, it was among the most digestible AA in PPI. The average CV associated with SID estimates of AA was highest in wcDDGS followed by corn and PPI.

The estimates of dLYS to optimize BWG (1.12%) were similar to the respective levels required to maximize FCR (1.13%). The dSAA levels to optimize BWG was 0.81% while no significant response was observe for FCR. The levels of dLYS required to optimize CY and levels of dLYS and dSAA required to optimize BMY, might be higher than corresponding levels required to optimize growth performance.

Recommendations for future research:

1. More studies on SID estimates of AA in PPI fed to broilers to utilize the high digestibility potential of PPI.
2. Methods of enhancing the AA digestibility, especially of lysine in DDGS.
3. Determine the requirement of dLYS for optimum CY and BMY in starters with levels higher than 1.30% of diet based upon IICP concept.

4. Determine the requirement of dSAA for optimum CY and BMY in starters with levels higher than 0.92% of diet based upon IICP concept.
5. Standardized method for comparing the results of different requirement studies in broilers.

8.0 REFERENCES

Acar, N., Jr Moran, E. T. and Bilgili, S. F. 1991. Live performance and carcass yield of male broilers from two commercial strain crosses receiving rations containing lysine below and above the established requirement between six and eight weeks of age. Poult. Sci. 70:2315-2321

Achinewhu, S. C., and Hewitt, D. 1979. Assessment of the nutritional quality of proteins: the use of 'ileal'digestibilities of amino acids as measures of their availabilities. Br. J. Nutr. 41:559-571.

Adedokun, S. A., Parsons, C. M., Lilburn, M. S., Adeola, O. and Applegate, T. J. 2007. Endogenous amino acid flow in broiler chicks is affected by the age of birds and method of estimation. Poult. Sci. 86:2590-2597.

Adedokun, S. A., Adeola, O., Parsons, C. M., Lilburn, M. S. and Applegate, T. J. 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult. Sci. 87:2535-2548.

Adedokun, S. A., Utterback, P., Parsons, C. M., Adeola, O., Lilburn, M. S. and Applegate, T. J. 2009. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caecectomised roosters. Br. Poult. Sci. 50:350-358.

Aftab, U., and Ashraf, M. 2009. Methionine plus cystine requirement of broiler chickens fed low-density diets under tropical conditions. Trop. Anim. Health Prod. 41:363-369.

Angkanaporn, K., Ravindran, V. and Bryden, W. L. 1996. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal, and meat and bone meal for broilers. Poult. Sci. 75:1098-1103.

Angkanaporn, K., Ravindran, V. and Bryden, W. L. 1997. Influence of caecectomy and dietary protein concentration on apparent excreta amino acid digestibility in adult cockerels. Br. Poult. Sci. 38:270-276.

AOAC.2000. Official Methods of Analysis. 17th ed. Assoc. Office.Anal.Chem., Arlington, VA.

AOAC.2005. Official Methods of Analysis. 18th ed. Assoc. Office.Anal.Chem., Arlington, VA

Baker, D. H. 1986. Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrients. J. Nutr. 116:2339-2349.

Baker, D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. Biokyowa Tech. Rev. 9:1-24.

Baker, D. H., and Han, Y. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult. Sci. 73:1441-1447.

Baker, D. H., Batal, A. B., Parr, T. M. N., Augspurger, R. and Parsons, C. M. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:485-494.

Bandegan, A. 2009. Standardized and apparent ileal digestibility of protein and amino acids in several feed ingredients fed to broilers. MSc. Thesis. University of Manitoba.

Bandegan, A., Guenter, W., Hoehler, D., Crow, G. H. and Nyachoti, C. M.. 2009. Standardized ileal amino acid digestibility in wheat distillers dried grains with solubles for broilers. Poult. Sci. 88:2592-2599.

Bandegan, A., Kiarie, E., Payne, R. L., Crow, G. H., Guenter, W. and Nyachoti, C. M. 2010. Standardized ileal amino acid digestibility in dry-extruded expelled soybean meal, extruded canola seed-pea, feather meal, and poultry by-product meal for broiler chickens. Poult. Sci. 89:2626-2633.

Batal, A. B., and Parsons, C. M. 2002. Effects of age on nutrient digestibility in chicks fed different diets. Poult. Sci. 81:400-407.

Batal, A. B., and Dale, N. M. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. J. Appl. Poult. Res. 15:89–93.

Batterham, E. S. 1992. Availability and utilization of amino acids for growing pigs. Nutr. Res. Rev. 5:1-18.

Batterham, E. S., Murison, R. D. and Lewis, C. E. 1979. Availability of lysine in protein concentrates as determined by the slope-ratio assay with growing pigs and rats and by chemical techniques. Br. J. Nutr. 41:383-391.

Bielorai, R., and Iosif, B. 1987. Amino acid absorption and endogenous amino acids in the lower ileum and excreta of chicks. J. Nutr. 117:1459-1462.

Bilgili, S. F., E. T. Moran Jr, and N. Acar. 1992. Strain cross response of heavy male broilers to dietary lysine in the finisher feed: live performance and further-processing yields. Poult. Sci. 71:850-858.

Borin, K., Ogle, B. and Lindberg, J. E. 2002. Methods and techniques for the determination of amino acid digestibility: A Review. Livest. Res. Rural Dev. (14)6

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. Can. Counc. Anim. Care, Ottawa, Ontario, Canada.

Chavan, U. D., Mckenzie, D. B. and Shahidi, F. 2001. Functional properties of protein isolates from beach pea (Lathyrus maritimus L.). Food Chem. 74:177-187.

Commission Directive. 1998. Establishing community methods for the determination of amino acids, crude oils and fats, and olanquidox in feeding stuff and amending Directive 71/39/EEC, annex part A. Determination of amino acids. Off. J. Eur. Communities L257:14-23.

Corzo, A., Jr Moran, E. T. and Hoehler, D. 2002. Lysine need of heavy broiler males applying the ideal protein concept. Poult. Sci. 81:1863-1868.

Corzo, A., Dozier, W. A. and Kidd, M. T. 2006. Dietary lysine needs of late-developing heavy broilers. Poult. Sci. 85:457-461.

Cowieson, A. J. 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Technol. 119:293-305.

Cromwell, G. L., Herkelman, K. L. and Stahly, T. H. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. J. Anim. Sci. 71:679-686.

Croy, R. R. D., Gatehouse, J. A., Tyler, M. L. and Boulter, D. 1980. The purification and characterization of a third storage protein (convicilin) from the seeds of pea (Pisum sativum L.). Biochem. J. 191:509-516.

Dari, R. L., Jr Penz, A. M., Kessler, A. M. and Jost, H. C. 2005. Use of digestible amino acids and the concept of ideal protein in feed formulation for broilers. J. Appl. Poult. Res. 14:195-203.

Degussa Corporation. 2006. AminoDat® 3.0 Platinum Print Version. Degussa AG, Feed additives. Hanau-Wolfang, Germany.

De Lange, C. F. M., Sauer, W. C., Mosenthin, R. and Souffrant, W. B. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. 67:746-754.

D'Mello, J. P. F. 1982. A comparison of two empirical methods of determining amino acid requirements. World Poult. Sci. J. 38:114-119.

D'Mello, J. P. F. 2003. Responses of growing poultry to amino acids. In J. P. F. D' Mello (2nd ed.) Amino Acids in Animal Nutrition. CAB Intl. Wallingford. pp 237-263.

Douglas, M. W., and Parsons, C. M. 1999. Dietary formulation with rendered spent hen meals on a total amino acid versus a digestible amino acid basis. Poult. Sci. 78:556-560.

Dozier III, W. A., Corzo, A., Kidd, M. T., Tillman, P. B. and Branton, S. L. 2009. Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age. Poult. Sci. 88:1676-1682.

Draper, N. R., and Smith, H. 1981. Applied Regression Analysis. 2nd ed. John Wiley and Sons. New York.

Emmert, J. L., and Baker, D. H. 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poult. Res. 6:462-470.

Evers, A. D., Blakeney, A. B. and O'Brien, L. 1999. Cereal structure and composition. Aust. J. Agric. Res. 50:629-650.

Fan, M. Z., and Sauer, W. C. 1995. Determination of apparent ileal amino acid digestibility in barley and canola meal for pigs with the direct, difference, and regression methods. J. Anim. Sci. 73:2364-2374.

Fan, M. Z., and Sauer, W. C. 1999. Variability of apparent ileal amino acid digestibility in different pea samples for growing-finishing pigs. Can. J. Anim. Sci. 79:467-475.

Fan, M. Z., Sauer, W. C., Hardin, R. T. and Lien, K. A. 1994. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. J. Anim. Sci. 72:2851-2859.

Farrell, D. J., Mannion, P. F., and Perez-Maldonado, R. A. 1999. A comparison of total and digestible amino acids in diets for broilers and layers. Anim. Feed Sci. and Tech. 82:131-142.

Fastinger, N. D., and Mahan, D. C. 2006. Determination of the ileal amino acid and energy digestibilities of corn distillers dried grains with solubles using grower-finisher pigs. J. Anim. Sci. 84:1722-1728.

Fastinger, N. D., Latshaw, J. D. and Mahan, D. C. 2006. Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poult. Sci. 85:1212-1216.

Feine, S. P., York, T. W. and Shasteen, C. 2006. Correlation of DDGS IDEA TM digestibility assay for poultry with cockerel true amino acid digestibility. Pages 82-89. In: Proc. 4th Mid-Atlantic Nutrition Conference. University of Maryland, College Park, MD.

Fernandez, S. R., Zhang, Y. and Parsons, C. M. 1995. Dietary formulation with cottonseed meal on a total amino acid versus a digestible amino acid basis. Poult. Sci. 74:1168-1179.

Friesen, M. J., Kiarie, E. and Nyachoti, C. M. 2006. Ileal amino acid digestibility and reactive lysine content in peas (Pisum sativum) fed to growing pigs. Anim. Feed Sci. Technol. 129:210-223.

Fuller, M. 2003. Amino acid bioavailability: A brief history. In: Digestive physiology in pigs. ed. R. O. Ball. 9TH Intl. Symp. Vol. 1. pp.183–198. Univ. Alberta, Alberta, Canada.

Furuya, S., and Kaji, Y. 1991. Additivity of the apparent and true ileal digestible amino acid supply in barley, maize, wheat or soya-bean meal based diets for growing pigs. Anim. Feed Sci. Technol. 32:321-331.

Gabriel, I., Lessire, M., Juin, H., Burstin, J., Duc, G., Quillien, L., Thibault, J. N., Leconte, M., Hallouis, J. M. and Ganier, P. 2008a. Variation in seed protein digestion of different pea (Pisum sativum L.) genotypes by cecectomized broiler chickens: 1. Endogenous amino acid losses, true digestibility and in vitro hydrolysis of proteins. Livest. Sci. 113:251-261.

Gabriel, I., Quillien, L., Cassecuelle, F., Marget, P., Juin, H., Lessire, M., Sève, B., Duc, G. and Burstin, J. 2008b. Variation in seed protein digestion of different pea (Pisum sativum L.) genotypes by cecectomized broiler chickens: 2. Relation between in vivo protein digestibility and pea seed characteristics, and identification of resistant pea polypeptides. Livest. Sci.113:262-273.

Garcia, A., and Batal, A. B. 2005. Changes in the digestible lysine and sulfur amino acid needs of broiler chicks during the first three weeks posthatching. Poult. Sci. 84:1350-1355.

Garcia, A., Batal, A. B. and Baker, D. H. 2006. Variations in the digestible lysine requirement of broiler chickens due to sex, performance parameters, rearing environment, and processing yield characteristics. Poult. Sci. 85:498-504.

Garcia, A., Batal, A. B. and Dale, N. M. 2007. A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. Poult. Sci. 86:94-101.

Gatel, F. 1994. Protein quality of legume seeds for non-ruminant animals: a literature review. Anim. Feed Sci. Technol. 45:317-348.

Golian, A., Guenter, W., Hoehler, D., Jahanian, H. and Nyachoti, C. M. 2008. Comparison of various methods for endogenous ileal amino acid flow determination in broiler chickens. Poult. Sci. 87:706-712.

Gorman, I., and Balnave, D. 1995. The effect of dietary lysine and methionine concentrations on the growth characteristics and breast meat yields of Australian broiler chickens. Aust. J. Agric. Res. 46:1569-1578.

Graber, G., and Baker, D. H. 1971. Sulfur amino acid nutrition of the growing chick: quantitative aspects concerning the efficacy of dietary methionine, cysteine and cystine. J. Anim. Sci. 33:1005-1011.

Green, S., Bertrand, S. L., Duron, M. J. C. and Maillard, R. 1987. Digestibilities of amino acids in maize, wheat and barley meals, determined with intact and caecectomised cockerels. Br. Poult. Sci. 28:631-641.

Grosjean, F., Jondreville, C., Williatte-Hazouard, I., Skiba, F., Carrouée, B. and Gatel, F. 2000. Ileal digestibility of protein and amino acids of feed peas with different trypsin inhibitor activity in pigs. Can. J. Anim. Sci. 80:643-652.

Gueguen, J. 1983. Legume seed protein extraction, processing, and end product characteristics. Plant Foods for Human Nutrition (Formerly Qualitas Plantarum) 32:267-303.

Gueguen, J., and Barbot, J. 1988. Quantitative and qualitative variability of pea (Pisum sativum L.) protein composition. J. Sci. Food Agric. 42:209-224.

Gurnsey, M. P., James, K. A. C. and Johns, D. C. 1985. An improved method of ileal cannulation of adult cockerels. Res. Vet. Sci. 39:390-391.

Han, Y. M., and Baker, D. H. 1991. Lysine requirements of fast- and slow-growing broiler chicks. Poult. Sci. 70:2108-2114.

Han, Y. M., and Baker, D. H. 1993. Effects of sex, heat stress, body weight, and genetic strain on the dietary lysine requirement of broiler chicks. Poult. Sci. 72:701-708.

Han, Y. M., and Baker, D. H. 1994. Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. Poult. Sci. 73:1739-1745.

Hickling, D., Guenter, W. and Jackson, M. E. 1990. The effects of dietary methionine and lysine on broiler chicken performance and breast meat yield. Can. J. Anim. Sci. 70:673-678.

Holsheimer, J. P., and Ruesink, E. W. 1993. Effect on performance, carcass composition, yield, and financial return of dietary energy and lysine levels in starter and finisher diets fed to broilers. Poult. Sci. 72:806-815.

Huang, K. H., Ravindran,V., Li, X. and Bryden, W. L. 2005. Influence of age on the apparent ileal amino acid digestibility of feed ingredients for broiler chickens. Br. Poult. Sci. 46:236-245.

Huang, K. H., X. Li, Ravindran, V. and Bryden, W. L. 2006. Comparison of apparent ileal amino acid digestibility of feed ingredients measured with broilers, layers, and roosters. Poult. Sci. 85:625.

Huyghebaert, G., and Pack, M. 1996. Effects of dietary protein content, addition of nonessential amino acids and dietary methionine to cysteine balance on responses to dietary sulphur-containing amino acids in broilers. Br. Poult. Sci. 37:623-639.

Igbasan, F. A., and Guenter, W. 1996. The evaluation and enhancement of the nutritive value of yellow-, green-and brown-seeded pea cultivars for unpelleted diets given to broiler chickens. Anim. Feed Sci. Technol. 63:9-24.

Igbasan, F. A., Guenter, W. and Slominski, B. A. 1997. Field peas: chemical composition and energy and amino acid availabilities for poultry. Can. J. Anim. Sci. 77:293-300.

Jensen, L. S., Wyatt, C. L. and Fancher, B. I. 1989. Sulfur amino acid requirement of broiler chickens from 3 to 6 weeks of age. Poult. Sci. 68:163-168.

Johns, D. C., Low, C. K., Sedcole, J. R. and James, K. A. C. 1986. Determination of amino acid digestibility using caecectomised and intact adult cockerels. Br. Poult. Sci. 27:451-461.

Johnson, E. J., and Brekke, C. A. 1983. Functional properties of acylated pea protein isolates. J. Food Sci. 48:722-725.

Johnson, R. J. 1992. Principles, problems and application of amino acid digestibility in poultry. Worlds Poult. Sci. J. 48:232-246.

Jondreville, C., Van den Broecke, J., Gâtel, F., Grosjean, F., Van Cauwenberghe, S. and Sève, B. 2001. Ileal digestibility of amino acids and estimates of endogenous amino acid losses in pigs fed wheat, triticale, rye, barley, maize and sorghum. Anim. Res. 50:119-134.

Kadim, I. T., and Moughan, P. J. 1997a. Development of an Ileal amino acid digestibility assay for the growing chicken—effects of time after feeding and site of sampling. Br. Poult. Sci. 38:89-95.

Kadim, I. T., and Moughan, P. J. 1997b. Ileal amino acid digestibility assay for the growing meat chicken—effect of the imposition of a fasting period and the nature of the test diet. Br. Poult. Sci. 38:285-290.

Kadim, I. T., Moughan, P. J. and Ravindran, V. 2002. Ileal amino acid digestibility assay for the growing meat chicken--comparison of ileal and excreta amino acid digestibility in the chicken. Br. Poult. Sci. 43:588-597.

Kalinowski, A., Jr Moran, E. T., and Wyatt, C. 2003. Methionine and cysteine requirements of slow-and fast-feathering male broilers from zero to three weeks of age. Poult. Sci. 82:1423-1427.

Kidd, M. T., and Fancher, B. I. 2001. Lysine needs of starting chicks and subsequent effects during the growing period. J. Appl. Poult. Res. 10:385-393.

Kidd, M. T., Kerr, B. J. and Anthony, N. B. 1997. Dietary interactions between lysine and threonine in broilers. Poult. Sci. 76:608-614.

Kidd, M. T., Kerr, B. J., Halpin, K. M., McWard, G. W. and Quarles, C. L. 1998. Lysine levels in starter and grower-finisher diets affect broiler performance and carcass traits. J. Appl. Poult. Res. 7:351-358.

Kerr, B. J., and Kidd, M. T. 1999. Amino acid supplementation of low-protein broiler diets: 2. Formulation on an ideal amino acid basis. J. Appl. Poult. Res. 8:310-320.

Kerr, B. J., Kidd, M. T., Halpin, K. M., McWard, G. W. and Quarles, C. L. 1999. Lysine level increases live performance and breast yield in male broilers. J. Appl. Poult. Res. 8:381-390.

Kluth, H., Mantei, M., Elwert, C. and Rodehutscord, M. 2005. Variation in precaecal amino acid and energy digestibility between pea (Pisum sativum) cultivars determined using a linear regression approach. Br. Poult. Sci. 46:325-332.

Knabe, D., LaRue, D., Gregg, E., Martinez, G and Jr Tanksley, T. 1989. Apparent digestibility of nitrogen and amino acids in protein feedstuffs by growing pigs. J. Anim. Sci. 67:441-458.

Knowles, T. A., and Southern, L. L. 1998. The lysine requirement and ratio of total sulfur amino acids to lysine for chicks fed adequate or inadequate lysine. Poult. Sci. 77:564-569.

Labadan Jr, M. C., Hsu, K. N. and Austic, R. C. 2001. Lysine and arginine requirements of broiler chickens at two-to three-week intervals to eight weeks of age. Poult. Sci. 80:599-606.

Lan, Y., Opapeju, F. O. and Nyachoti, C. M. 2008. True ileal protein and amino acid digestibilities in wheat dried distillers' grains with solubles fed to finishing pigs. Anim. Feed Sci. Technol. 140:155-163.

Laplace, J., Darcy-Vrillon, B., Perez, J. M., Henry, Y., Giger, S. and Sauvant, D. 1989. Associative effects between two fibre sources on ileal and overall digestibilities of amino acids, energy and cell-wall components in growing pigs. Br. J. Nutr. 61:75-87.

Leclercq, B. 1998. Lysine: Specific effects of lysine on broiler production: comparison with threonine and valine. Poult. Sci. 77:118-123.

Le Guen, M. P., Huisman,J., Gueguen, J., Beelen, G. and Verstegen, M. W. A. 1995. Effects of a concentrate of pea antinutritional factors on pea protein digestibility in piglets. Livest. Prod. Sci. 44:157-167.

Lemme, A., Ravindran, V. and Bryden, W. L. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. Worlds Poult. Sci. J. 60:423-438.

Llames, C., and Fontaine, J. 1994. Determination of amino acids in feeds: Collaborative study. J. AOAC Int. 77:1362-1402.

Lin, F. D., Knabe, D. A. and Jr Tanksley, T. D. 1987. Apparent digestibility of amino acids, gross energy and starch in corn, sorghum, wheat, barley, oat groats and wheat middlings for growing pigs. J Anim. Sci. 64:1655-1665.

Low, A. G. 1980. Nutrient absorption in pigs. J. Sci. Food Agric. 31:1087-1130.

Lumpkins, B. S., and Batal, A. B. 2005. The bioavailability of lysine and phosphorus in distillers dried grains with solubles. Poult. Sci. 84:581-586.

Lumpkins, B. S., Batal, A. B. and Baker, D. H. 2007. Variations in the digestible sulfur amino acid requirement of broiler chickens due to sex, growth criteria, rearing environment, and processing yield characteristics. Poult. Sci. 86:325-330.

Lumpkins, B. S., Batal, A. B. and Dale, N. M. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. Poult. Sci. 83:1891-1896.

Mack, S. D., Bercovici, D., De Groote, G. E., Leclercq, B., Lippens, M., Pack, M., Schutte, J. S. and Van Cauwenberghe, S. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. Br. Poult. Sci. 40:257-265.

Martinez-Amezcua, C., Parsons, C. M., Singh, V., Srinivasan, R. and Murthy, G. 2007. Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. Poult. Sci. 86:2624-2630.

Mauron, J. 1981. The maillard reaction in food; A critical review from the nutritional standpoint. Prog. Food Nutr. Sci. 5:5-35.

McNab, J. M. 1994. Amino acid digestibility and availability studies with poultry. In: Farm Animal Nutrition, ed. by D' Mello J. P. F.(CAB International). pp 185-203.

Mendonça, C. X., and Jensen, L. S. 1989. Influence of protein concentration on the sulphur-containing amino acid requirement of broiler chickens. Br. Poult. Sci. 30:889-898.

Moran Jr, E. T., and Bilgili, S. F. 1990. Processing losses, carcass quality, and meat yields of broiler chickens receiving diets marginally deficient to adequate in lysine prior to marketing. Poult. Sci. 69:702-709.

Morris, T. R., Gous, R. M. and Abebe, S. 1992. Effects of dietary protein concentration on the response of growing chicks to methionine. Br. Poult. Sci. 33:795-803.

Mosenthin, R., Sauer, W. C., Blank, R., Huisman, J. and Fan, M. Z. 2000. The concept of digestible amino acids in diet formulation for pigs. Livest. Prod. Sci. 64:265-280.

Moughan, P. J. 2003. Amino acid availability: aspects of chemical analysis and bioassay methodology. Nutr. Res. Rev.16:127-141.

Nouri-Emamzadeh, A., and A. Yaghobfar. 2009. Influence of caecectomy on digestibility of amino acids for soybean, canola and sunflower meals in adult cockerels. J. Appl. Poult. Res. 46:19-24.

Nouri-Emamzadeh, A., Yaghobfar, A., Sadeghi, A., Mirhadi, A. and Chamani, M. 2009. Influence of caecectomy on endogenous amino acid losses in adult cockerels. J. Anim. Vet. Adv 8:108-110.

Noy, Y., and Sklan, D. 1995. Digestion and absorption in the young chick. Poult. Sci. 74:366-373.

NRC, 1994. (National Research Council), Nutrient requirements of domestic animals. Nutrient requirements of poultry (9th ed.) National Academy Press, Washington, DC.

Nyachoti, C. M, De Lange, C. F. M., Mcbride, B.W. and Schulze, H. 1997. Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: a review. Can. J. Anim. Sci. 77:149-163.

Nyachoti, C. M., House, J. D., Slominski, B. A. and Seddon, I. R. 2005. Energy and nutrient digestibilities in wheat dried distillers' grains with solubles fed to growing pigs. J. Sci. Food Agric. 85:2581-2586.

Opapeju, F. O., Nyachoti, C. M. and House, J. D. 2007. Digestible energy, protein and amino acid content in selected short season corn cultivars fed to growing pigs. Can. J. Anim. Sci. 87:221-226.

Owusu-Asiedu, A., Baidoo, S. K. and Nyachoti, C. M. 2002. Effect of heat processing on nutrient digestibility in pea and supplementing amylase and xylanase to raw, extruded or micronized pea-based diets on performance of early-weaned pigs. Can. J. Anim. Sci. 82:367-374.

Parsons, C. M. 1984. Influence of caecectomy and source of dietary fibre or starch on excretion of endogenous amino acids by laying hens. Br. J. Nutr. 51:541-548.

Parsons, C. M. 1985. Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. J. Agri. Sci. 104:469-472.

Parsons, C. M. 1986. Determination of digestible and available amino acids in meat meal using conventional and caecectomized cockerels or chick growth assays. Br. J. Nutr. 56:227-240.

Parsons, C. M., Potter, L. M. and Jr Brown, R. D. 1983a. Effects of dietary carbohydrate and of intestinal microflora on excretion of endogenous amino acids by poultry. Poult. Sci. 62:483-489.

Parsons, C. M., Baker, D. H. and Harter, J. M. 1983b. Distillers dried grains with solubles as a protein source for the chick. Poult. Sci. 62:2445-2451.

Parsons, C. M., Potter, L. M. and Jr Brown, R. D. 1981. True metabolizable energy and amino acid digestibility of dehulled soybean meal. Poult. Sci. 60:2687-2696.

Parsons, C. M., Potter, L. M. and Jr Brown, R. D. 1982. Effects of dietary protein and intestinal microflora on excretion of amino acids in poultry. Poult. Sci. 61:939-946.

Parsons, C. M., Hashimoto, K., Wedekind, K. J., Han, Y. and Baker, D. H. 1992. Effect of overprocessing on availability of amino acids and energy in soybean meal. Poult. Sci. 71:133-140.

Payne, W. L., Combs, G. F., Kifer, R. R. and Snyder, D. G. 1968. Investigation of protein quality--ileal recovery of amino acids. Fed. Proc. 27:1199-1203.

Raharjo, Y. C., and Farrell, D. J. 1984. A new biological method for determining amino acid digestibility in poultry feedstuffs using a simple cannula, and the influence of dietary fibre on endogenous amino acid output. Anim. Feed Sci. Technol. 12:29-45.

Ravindran, V., and Bryden, W. L. 1999. Amino acid availability in poultry-in vitro and in vivo measurements. Aust. J. Agric. Res. 50:889-908.

Ravindran, V., and Hendriks, W. H. 2004. Endogenous amino acid flows at the terminal ileum of broilers, layers and adult roosters. Anim. Sci. 79:265-272.

Ravindran, V., Hew, L. I., Ravindran, G. and Bryden, W. L. 1999. A comparison of ileal digesta and excreta analysis for the determination of amino acid digestibility in food ingredients for poultry. Br. Poult. Sci. 40:266-274.

Ravindran, V., Hew, L. I., Ravindran, G. and Bryden, W. L. 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. Anim. Sci. 81:85-97.

Renden, J. A., Jr Moran, E. T. and Kincaid, S. A. 1994. Lack of interactions between dietary lysine or strain cross and photoschedule for male broiler performance and carcass yield. Poult. Sci. 73:1651-1662.

Robbins, K. R., Norton, H. W. and Baker, D. H. 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109:1710-1714.

Rodehutscord, M., and Pack, M. 1999. Estimates of essential amino acid requirements from dose-response studies with rainbow trout and broiler chicken: Effect of mathematical model. Arch. of Anim. Nutr. 52:223-244.

Rostagno, H. S., Pupa, J. M. R. and Pack, M. 1995. Diet formulation for broilers based on total versus digestible amino acids. J. Appl. Poult. Res. 4:293.

Runnels, T. D. 1966. The biological nutrient availability of corn distillers dried grains with solubles in broiler feeds. Proc. Distillers Feed Research Council 21:11-15. Cincinnati, OH.

Runnels, T. D. 1968. Effective levels of distillers feeds in poultry rations. Proc. Distillers Res. Council. 23:15-22, Cincinnati, OH.

Sakata, T. 1987. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. Br. J. Nutr. 58:95-103.

Salter, D. N., and Fulford, R. J. 1974. The influence of the gut microflora on the digestion of dietary and endogenous proteins: studies of the amino acid composition of the excreta of germ-free and conventional chicks. Br. J. Nutr. 32:625-637.

SAS Institute. 1990. SAS User's Guide: Statistics. SAS Inst.Inc., Cary, NC.

Sauer, W. C., and Ozimek, L. 1986. Digestibility of amino acids in swine: results and their practical applications. A review. Livest. Prod. Sci. 15:367-388.

Sauer, W., Stothers, S. and Parker, R. 1977. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57:775-784.

Schutte, J. B., and Pack, M. 1995a. Effects of dietary sulphur-containing amino acids on performance and breast meat deposition of broiler chicks during the growing and finishing phases. Br. Poult. Sci. 36:747-762.

Schutte, J. B., and Pack, M. 1995b. Sulfur amino acid requirement of broiler chicks from 14 to 38 days of age. 1. Performance and carcass yield. Poult. Sci. 74:480-487.

Short, F. J., Wiseman, J. and Boorman, K. N. 1999. Application of a method to determine ileal digestibility in broilers of amino acids in wheat. Anim. Feed Sci. Technol. 79:195-209.

Sibbald , I. R. 1979. A bioassay for available amino acids and true metabolizable energy in feedingstuffs. Poult. Sci. 58:668-675.

Sibbald, I. R. 1987. Estimation of bioavailable amino acids in feedingstuffs for poultry and pigs: a review with emphasis on balance experiments. Can. J. Anim. Sci. 67:221-300.

Sibbald, I. R., and Wolynetz, M. S. 1986. Effects of dietary lysine and feed intake on energy utilization and tissue synthesis by broiler chicks. Poult. Sci. 65:98-105.

Sibbald, I. R., and Wolynetz, M. S. 1987. Effects of dietary fat level and lysine:energy ratio on energy utilization and tissue synthesis by broiler chicks. Poult. Sci. 66:1788-1797.

Siriwan, P., Bryden, W. L., Mollah, Y. and Annison, E. F. 1993. Measurement of endogenous amino acid losses in poultry. Br. Poult. Sci. 34:939-949.

Sklan, D., and Noy, Y. 2003. Crude protein and essential amino acid requirements in chicks during the first week posthatch. Br. Poult. Sci. 44:266-274.

Song, G. L., Li, D. F., Piao, X., Chi, F., Chen,Y. and Moughan, P. J. 2004. True amino acid availability in chinese high-oil corn varieties determined in two types of chickens. Poult. Sci. 83:683-688.

Spiehs, M. J., Whitney, M. H. and Shurson, G. C. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639-2645.

Stein, H. H., Seve, B., Fuller, M. F., Moughan, P. J. and De Lange, C. F. M. 2007a. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J. Anim. Sci. 85:172-180.

Stein, H. H., Pedersen, C., Wirt, A. and Bohlke, R. A. 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J. Anim. Sci. 83:2387-2395.

Stein, H. H., and Bohlke, R. A. 2007. The effects of thermal treatment of field peas (Pisum sativum) on nutrient and energy digestibility by growing pigs. J. Anim. Sci. 1424-1431.

Stein, H. H., Fuller, M. F., Moughan, P. J, Sève, B., Mosenthin, R. A., Jansman, J. M., Fernández, J. A. and De Lange, C. F. M. 2007b. Definition of apparent, true, and standardized ileal digestibility of amino acids in pigs. Livest. Sci. 109:282-285.

Stein, H. H., Gibson, M. L., Pedersen, C. and Boersma, M. G. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. J. Anim. Sci. 84:853-860.

Stein, H. H., Kim, S. W., Nielsen, T. T. and Easter, R. A. 2001. Standardized ileal protein and amino acid digestibility by growing pigs and sows. J. Anim. Sci. 79:2113-2122.

Summers, J. D., and S. Leeson. 1985. Broiler carcass composition as affected by amino acid supplementation. Can. J. Anim. Sci. 65:717-723.

Świątkiewicz, S., and J. Koreleski. 2008. The use of distillers dried grains with soluble (DDGS) in poultry nutrition. Worlds Poult. Sci. 64:257-266.

Taherkhani, R., Shivazad, M., Zaghari, M. and Shahneh, A. Z. 2005. Male and Female Broilers response to different ideal amino acid ratios during the second and third weeks posthatch. Intl. J. Poult. Sci. 4:563-567.

Taherkhani, R., Shivazad, M., Zaghari, M. and Shahneh, A. Z. 2008. Comparison of different ideal amino acid ratios in male and female broiler chickens of 21 to 42 days of age. J. Poult. Sci. 45:15-19.

Tanksley Jr, T. D., Knabe, D. A., Purser, K., Zebrowska, T. and Corley, J. R. 1981. Apparent digestibility of amino acids and nitrogen in three cottonseed meals and one soybean meal. J. Anim. Sci. 52:769-777.

Ten Doeschate, R. A. H. M., Scheele, C. W., Schreurs,V. V. A. M. and Van Der Klis, J. D. 1993. Digestibility studies in broiler chickens: influence of genotype, age, sex and method of determination. Br. Poult. Sci. 34:131-146.

Thacker, P. A., and Widyaratne, G. P. 2007. Nutritional value of diets containing graded levels of wheat distillers grains with solubles fed to broiler chicks. J. Sci. Food Agric. 87:1386-1390.

Urriola, P. E., Hoehler, D., Pedersen, C., Stein, H. H. and Shurson, G. C. 2009. Amino acid digestibility of distillers dried grains with solubles, produced from sorghum, a sorghum-corn blend, and corn fed to growing pigs. J. Anim. Sci. 87:2574-2580.

Valencia, D. G., Serrano, M. P., Centeno, C., Lázaro, R. and Mateos, G. G. 2008. Pea protein as a substitute of soya bean protein in diets for young pigs: Effects on productivity and digestive traits. Livest. Sci.118:1-10.

Valencia, D. G., Serrano, M. P., Jiménez-Moreno, E., Lázaro, R. and Mateos, G. G. 2009. Ileal digestibility of amino acids of pea protein concentrate and soya protein sources in broiler chicks. Livest. Sci. 121:21-27.

Van Leeuwen, P., Sauer, W. C., Huisman, J., Van Weerden, E. J., Van Kleef, D. and Den Hartog, L. A. 1987. Methodological aspects for the determination of amino acid digestibilities in pigs fitted with ileo-cecal re-entrant cannulas. J. Anim. Physiol. Anim. Nutr. 58:122-133.

Vazquez, M., and Pesti, G. M. 1997. Estimation of the lysine requirement of broiler chicks for maximum body gain and feed efficiency. Livest. Sci. 6:241-246.

Vedenov, D., and Pesti, G. M. 2008. A comparison of methods of fitting several models to nutritional response data. J. Anim. Sci.86:500-507.

Vieira, S. L., Lemme, A., Goldenberg, D. B. and Brugalli, I. 2004. Responses of growing broilers to diets with increased sulfur amino acids to lysine ratios at two dietary protein levels. Poult. Sci. 83:1307-1313.

Waldroup, P. W., Owen, J. A., Ramsey, B. E. and Whelchel, D. L. 1981. The use of high levels of distillers dried grains plus solubles in broiler diets. Poult. Sci. 60:1479-1484.

Wanasundara, P., and Shahidi, F. 1997. Functional properties of acylated flax protein isolates. J. Agric. Food Chem. 45:2431-2441.

Wang, T. C., and Fuller, M. F. 1989. The optimum dietary amino acid pattern for growing pigs. Br. J. Nutr. 62:77-89.

Wang, X., and Parsons, C. M. 1998. Dietary formulation with meat and bone meal on a total versus a digestible or bioavailable amino acid basis. Poult. Sci. 77:1010-1015.

Wang, Z., Cerrate, S., Coto, Yan, C. F. and Waldroup P. W. 2007. Use of constant or increasing levels of distillers dried grains with solubles (DDGS) in broiler diets. Int. J. Poult. Sci 6:501-507.

Webb Jr, K. 1990. Intestinal absorption of protein hydrolysis products: a review. J. Anim. Sci. 68:3011-3022.

Whitacre, M. E., and Tanner, H. 1989. Methods of determining the bioavailability of amino acids for poultry. In Friedman, M. (Ed.) Absorption and Utilization of Amino Acids, Vol. III (CRC Press), Florida. pp 129-141.

Wiseman, J., Al-Mazooqi, W., Welham, T and Domoney, C. 2003. The apparent ileal digestibility, determined with young broilers, of amino acids in near-isogenic lines of peas (Pisum sativum L) differing in trypsin inhibitor activity. J. Sci. Food Agric. 83:644-651.

Youssef, I. M. I., Westfahla,C. , Sunderb, A., Liebertb, F. and Kamphuesa, J. 2008. Evaluation of dried distillers' grains with solubles (DDGS) as a protein source for broilers. Arch. Anim. Nutr. 62:404-414.

Zaghari, M., Shivazad, M., Kamyab, A. and Nikkhah, A. 2002. Digestible lysine requirement of arian male and female broiler chicks during six to twenty-one days of age. J. Agric. Sci. 4:111-117.

9.0 APPENDIX

Table 9.1. Basal ileal endogenous AA flow (mg/kg DM intake) in chicks fed a nitrogen-free diet

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

Adapted and modified from Golian et al. (2008)

Table 9.2. Estimated requirements based on quadratic regression analysis of digestible lysine (%) and digestible SAA (%) in 3-week old broilers

Amino acid (% of diet)	Response criteria	Equation[1]	R^2	Requirement estimates
Digestible Lysine	BW gain, g	-3269.7 + 6896.6 × (lysine) - 3060.5 × (lysine × lysine)	0.82	1.12
	FCR, g/g	13.137 – 19.462 × (lysine) + 8.5727 × (lysine × lysine)	0.85	1.13
Digestible SAA	BW gain, g	-1933.5 + 6284 × (SAA) - 3546.5 × (SAA × SAA)	0.40	0.89

[1]Prediction equation based on determined digestible lysine or digestible SAA for optimum response.