STRATEGIES TO IMPROVE POULTRY FEED FORMULATION FOR MAXIMUM PERFORMANCE AND PROFITABILITY

by

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ABSTRACT

A comprehensive research project was carried out in an attempt to improve poultry feed formulation using different strategies. In study 1, a simulation analysis in Excel was conducted to evaluate the batch segregation as a means to reduce nutrient variability for linear and non-linear feed formulations. batch segregation reduced variability about 50% compared to the traditional method of no segregation. Non-linear formulation increased feed costs to guarantee the minimum specification of nutrients at any confidence level. Employing the batch segregation when using non-linear formulation resulted in reducing feed costs. In study 2, A meta-analysis was conducted to quantify the optimal balance between dlys & true protein (TP) levels in broiler feeds to account for the needs of the NEAAs during formulation. The dlys requirements increased linearly as a function of TP. For maximum BWG, the dLys requirement was estimated to be 4.92% ± 0.51 of TP. In study 3, a 35-d broiler trial was conducted to test whether or not feeds formulated based on digestible AA values from chick or rooster assays could make differences in performance and profitability. Formulation based on chick assay resulted in improved FCR compared to rooster assay. Profitability varied depending on feed cost, chicken value and size. In study 4, a simulation analysis was conducted in Excel to test the effectiveness of broken- line linear (BLL) and broken-line quadratic (BLQ) models in estimating the maximum safe level (MSL) of feedstuffs in lieu of the traditional multiple range procedure. The broken-line methodology provided good estimates of the MSL (small SE and high R^2 values) and offered useful information for feeding trial planning. In study 5, 2 broiler trials were conducted to evaluate the nutritive value of pennycress meal (PM) as a protein source for broilers and to illustrate how the MSL can be different depending on the statistical analysis. The MSL was estimated in trial 1 to be 10% (orthogonal contrast), 9.12 ± 0.50 (BLL) and 7.0 ± 1.27 (BLQ). In trial 2, the estimated MSL was 12% (contrast and LSD), 15% (Scheffe's), 10.84 ± 0.57 (BLL) and 8.61 ± 1.29 by (BLQ).

INDEX WORDS: Batch segregation, Simulation, Broiler, Feed formulation, True protein, Amino acid, Broken line, Safe level

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DEDICATION

To my parents, wife and kids. Thank you all for your unconditional love and support.

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CHAPTER 1

INTRODUCTION

Poultry production has experienced a tremendous improvement in the past few decades. This great improvement encompassed almost all aspects of poultry production such as nutrition and genetic selection. Nutrition is central in poultry production because of its direct influence on performance and production economics. Feed formulation is the applied side of nutrition where nutritionists apply their knowledges to meet the nutritional requirements by formulating more economical feeds for maximum performance. Feed cost accounts for at least 60% of the total production costs in poultry production. Therefore, further improvements in the feed formulation process would maximize performance and profitability in poultry production.

One way to improve feed formulation is to improve feed quality by reducing nutrient variability. Considerable nutrient variability can result in under-feeding or over-feeding of essential nutrients, resulting in reduced bird performance, added input costs, and increased environmental pollution (Duncan, 1988). A few solutions have been proposed to deal with nutrient variability in feedstuffs since recognizing this problem in the 1960s. Margin of safety inclusion and non-linear “stochastic” feed formulation are the most important solutions proposed (Nott and Combs, 1967; Rahman and Bender,1971).

Another possible way to improve feed formulation is by accounting for the requirements of the essential (**EAAs**) and non-essential (**NEAAs**) amino acids in feed, especially in low protein diets. NEAAs are required for protein synthesis in the body as the EAAs, therefore a source of these amino acids must be present in adequate quantities. The source can be the NEAAs themselves or the excesses of the EAAs as a source of amino nitrogen (Heger, 2003). Typically, poultry feed is formulated to meet the minimum specifications of some of the EAAs, ignoring the need of providing enough levels of the NEAAs (or a source of amino nitrogen) for maximum performance. Several ways have been proposed to account for the NEAAs or amino nitrogen requirements during feed formulation like estimating the relationship between the EAA and NEAA (Young and Zamora, 1968), EAA and total AA (Ikemoto et al., 1989), EAA nitrogen and NEAA nitrogen (Lenis et al., 1999) and EAA nitrogen and total nitrogen (Evonik, 2005).

Feed formulation can also be improved by estimating the maximum safe (inclusion) level (**MSL**) of feed ingredients accurately. Potential and new cultivars of feed ingredients can contain limiting factors (e.g. anti-nutritional factors, fiber, AA profile, etc.) when fed at high levels. Therefore, these ingredients are evaluated in feeding trials involving feeding increasing levels of the test ingredient and eventually measuring the response (e.g. growth). Typically, a multiple range test (**MRT**) is used to define the MSL statistically (Jankowski et al., 2003; Hidalgo et al., 2004; Baurhoo et al., 2011). From a statistical stand point, using a MRT to estimate the MSL of ingredients is inappropriate for several reasons (Petersen, 1977; Dawkins, 1983; Lowry; 1992; Pesti et al., 2009). First, the levels of the input factor (test ingredient), which is continuous variable, are assumed to be discrete by the MRT. Second, The MRT distinguishes between two levels of the factor (no exact estimate). finally, the power of the MRT varies depending on the test. For example, Scheffe’s test (Scheffe, 1953) is a very conservative test and result in fewer differences while Fisher’s LSD test (Fisher, 1935) is a less conservative tests and can result in false differences. Therefore, there is a need for more precise methodologies to estimate the MSL.

Lastly, since protein is an expensive nutrient to feed, choosing the right digestible amino acid values can improve the feed formulation process. Typically, digestible amino acids are based on the precision-fed rooster assay (Parson, 1985) and the standardized ileal digestibility chick assay (Lemme et al., 2004). Digestibility data for common feedstuffs obtained using both assays are now available. Ajinomoto Heartland Inc. (AHI; Chicago, IL; rooster assay) and Evonik Industries (ED; Hanau-Wolfgang, Germany; chick assay) are the major amino acid databases supplying these data.

The objectives of the current dissertation were as follows:

1) To compare two grain handling techniques and two feed formulation methods (linear versus stochastic programming) to reduce crude protein variability in finished feeds and determine resulting costs or savings. The two grain handling techniques were placing all the random batches of each delivered ingredient in to 1) a single bin (1-bin method), or 2) segregating above and below average samples into two bins (2-bin method).
2) To investigate the relationship between digestible lysine (dLys) requirements to true protein for data compiled from literature on lysine requirements of broilers; and to develop prediction equations for the requirements of dLys for broiler chickens as a function of true protein that can be used in feed formulation to represent the requirements of NEAAs.
3) To evaluate the effectiveness of broken-line linear (**BLL**) and the broken-line quadratic (**BLQ**) models in estimating the MSL of feed ingredients using simulations in Microsoft Excel; and to examine the effect of the experiment design parameters (such as number of replications, ingredient levels and the number of simulations during the simulation

process when planning feeding trials) on the estimated parameters and their descriptive statistics

4) To evaluate the performance and processing yields of male broilers fed diets formulated on the basis of digestible AA values obtained from either AHI or ED database and to conduct an economic comparison of the two databases.

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CHAPTER 2

LITERATURE REVIEW

I. BASICS OF FEED FORMULATION

Feed Formulation

Feed formulation is the process of finding the optimal combination of feed ingredients that meets the specified nutritional requirements for specific types of poultry. Feed formulation is an applied field of nutrition where nutritionists apply their knowledge to produce a balanced diet. Many pieces of information should be incorporated prior to feed formulation to maximize the efficiency of formulation. Information like nutritional requirements, feedstuffs composition, nutrient availability and costs of available feedstuffs are essential elements for effective feed formulation. There are several techniques used to formulate poultry feeds. Hand formulation was practiced for long time before the emergence of modern computers with the capability of solving complex mathematical problems in 1950s.

Feed Formulation Techniques

Linear Programming

Typically, feed formulation is based on linear programming (**LP**) and referred to as least cost feed formulation. One of the earliest reports on using LP to formulate a least cost feed for poultry dates back to 1960 when Potter et al. (1960) used an IBM 704 computer at Massachusetts Institute of Technology Computation Center (Cambridge, MA) to formulate broiler diets. The formulation based on LP gained acceptance thereafter and become a helpful tool in feed formulation (Muller, 1971; Waldroup, 1973). Commercial companies have developed a great number of software capable of solving linear equations and varying in complexity from sophisticated programs to a simple Microsoft Excel worksheet (Redmond, WA). The LP model can handle very complicated situations where there are several nutrients to be met and multiple ingredients to choose from by solving a series of equations or inequalities (constraints) simultaneously. The LP model can be represented by the following mathematical constraints as detailed by D'Alfonso et al. (1992):

Minimize $\sum_{j=1}^{n} c_j x_j$ [1]

Subject to

$\sum_{j=1}^{n} a_{ij} x_j \geq b_i$ [2]

For i = 1,…., p.

$\sum_{j=1}^{n} a_{ij} x_j \leq b_i$ [3]

For I = p+1, p+2,…, m.

$\sum_{j=1}^{n} x_j = 1$ [4]

$x_j \geq 0$ [5]

For j = 1, …, n.

Where

j is one of the feed ingredients comprising the finished feed

n is the total number of feed ingredients

x_j is the fraction of the j^{th} feed ingredient

c_j is the cost of the fraction of the j^{th} feed ingredient

a_i the concentration of the i^{th} nutrient

b_i the minimum or maximum specification of the i^{th} nutrient

Feed formulation using linear programing model is usually based on data obtained from ingredient composition tables. The nutrient compositions in these table are averages not actual values. As a result, the chance of meeting the nutritional requirements is only 50% of the time in this case. To increase the confidence level of meeting the nutritional requirements to higher level an approach called non-linear programing should be used.

Nonlinear Programing

Non-linear programing or stochastic programming (**SP**) is an optimization approach based on probability distribution proposed to deal with situations (e.g. nutrient variability) where uncertainty is involved. This approach can be used to assure meeting the requirement of a nutrient during feed formulation at certain confidence levels by providing adjusted margin of safety of the nutrient. The stochastic programming approach takes the variability of nutrients into account during the feed formulation process by using the standard deviations of nutrients in feed ingredients during the optimization process. Derivation of the stochastic function from linear functions was detailed (D'Alfonso et al., 1992). To increase the probability (P) of meeting the requirement of a nutrient from 50% as with the LP model to a higher probability rate (α_k), constraint [2] should be converted to:

$$P\left(\sum_{j=1}^{n} a_{kj}\, x_j \;\; \geq b_k\right) \geq \alpha_k \quad [6]$$

And constraint [3] should be converted to:

$P\left(\sum_{j=1}^{n} a_{kj}\, x_j \;\; \leq b_k\right) \geq \alpha_k$ [7]

The term $P(A) \geq \alpha_k$ indicates the probability of event A happening (i.e. meeting a nutrient specification) is equal or greater than a certain probability value between 0 to 1. To account for nutrient variability, constraints [6] and [7] should be modified. Provided that nutrient levels in feed ingredients (a_{ij}) are independent and normally distributed random variables with a mean u_{ij} and standard deviation σ_{ij}, constraints [6] can be simplified to;

$\sum_{j=1}^{n} u_{ij}\, x_j \; + \; Z_i \; \sqrt{\sum_{j=1}^{n} \sigma_{ij}^2\, x_J^2} \; \geq b_i$ [8]

And constraint [7] can be simplified to:

$\sum_{j=1}^{n} u_{ij}\, x_j \; + \; Z_i \; \sqrt{\sum_{j=1}^{n} \sigma_{ij}^2\, x_J^2} \; \leq b_i$ [9]

Where Z_i is the standard normal deviate corresponding to α_i,

$Z_i \leq 0$ for $\alpha_i \geq 0.50$ in constraints [8] and $Z_i \geq 0$ for $\alpha_i \geq 0.50$ in constraints [9]

Both [8] and [9] are nonlinear constraints representing the stochastic model. Reports comparing LP with a margin of safety and SP suggested economic feasibility of using the SP in formulating poultry diets (D'Alfonso and Roush, 1990; D’Alfonso et al., 1992; D’Alfonso et al., 1993; Cravener et al., 1994).

Hand Formulation

Simple diets with a very few ingredients and nutrients could be formulated (balanced) using hand formulation techniques such as simple equations or Pearson’s square. Usually, these simple techniques are used to meet the requirements of two nutrients utilizing only two feed ingredients. The price of feed is not taken into account when using these techniques because it is difficult to achieve the cheapest feed here. Nowadays, the availability of personal computers and feed formulation software have eliminated the need to formulate feeds by hand calculation.

II. ELEMENTS OF FEED FORMULATION

Feed Ingredients

In the beginning of the 20th century, a few feedstuffs such as corn, wheat, meat scrap and milk products were available for poultry (Nixon, 1915; Rogers, 1915). Nowadays, the feed industry is faced with a wide selection of feedstuffs including but are not limited to by-products of oil production, cereal by-products, animal by-products and synthetic amino acids. Several factors decide selecting ingredients for feed formulation including ingredient availability, price, and the quality of the composing nutrients. Thus, corn and soybean meal have become the standard ingredients used in poultry feeds. Feed ingredients supply the nutritional requirements of energy, protein (amino acids), vitamins and minerals. To meet the nutritional requirements when formulating poultry feeds, the nutrient composition of the ingredients must be known and this can be achieved by laboratory analysis.

Nutrient Composition of Feed Ingredients

Typically, nutritionists and feed formulators base their formulations on data obtained from ingredient composition tables because it is impractical to analyze the nutrient content of each batch of feed. The nutrient levels reported in these tables are averages originated from many laboratory analyses. The Subcommittee on Poultry Nutrition of the National Research Council (NRC. 1994) compiled data from several sources on the nutritional composition of key ingredient commonly used in poultry feeds. The data included the contents of key nutrients required by poultry (as fed-basis) such as metabolizable energy, protein and total amino acids. Although the use of digestible amino acids at that time was not common, the true digestibility coefficients of amino acids for selected ingredient were reported. In recent years, the use of digestible amino acids as estimated by digestibility assays replaced the use of total amino acids in feed formulation models in most parts of the world. Understanding AAs utilization by the birds is a necessary step that nutritionist should take into account before formulating broiler diets. The AAs utilization can be evaluated in vivo using digestibility assays.

Digestibility Assays for Amino Acids

Formulating poultry diets on the basis of total amino acids (AAs) has been practiced for long time. Amino acids in raw materials are not totally digested by the birds as variable portions of these vital compounds are undigested and unavailable to the birds. To avoid any potential reduction in performance resulting from formulating diets based on total AAs of ingredients characterized with low AAs digestibility, a margin of safety for AAs was necessary. Nutritionist and feed formulators in many parts of the world realized the necessity to shift toward formulating diets on the basis of digestible AAs so the AAs requirements are met more closely. The purpose of this shift was primarily to minimize AAs overfeeding and feed costs to maximize economic returns and to minimize environmental pollution. To obtain information on AAs digestibility for a feed ingredient, a known amount of this test ingredient has to be fed alone or in a balanced diet (test ingredient is the only source of AAs) in a digestibility trial and then the digestibility coefficient is determined of each AA (Digestibility Coefficient (%) = ((AA_{Diet} – $AA_{Excreta}$) /$AA_{Diet)}$ x 100). AA digestibility can be classified into two categories based on the age of the bird used. There are two main categories of AA digestibility assays that can be used to determine the digestibility of AAs in feedstuffs, the precision-fed rooster assay and the chick assays.

The precision-fed rooster assay or simply rooster assay was based on the true metabolizable energy (TME) assay developed by Sibbald (Sibbald, 1976). Likuski and Dorrell (1978) were first to adapt Sibbald rooster assay for TME to determine AA digestibility in samples of corn and soybean meal. The rooster assay as described by Sibbald (Sibbald, 1986) involves force-feeding mature roosters (intact or cecectomized) known amounts of the test ingredient via a tube after fasting the roosters for a period of time to empty their gut then collecting the excreta over the next 48 hrs for AA analysis. To correct for endogenous amino acid excretion, the test roosters should be compared to fasting rosters or roosters fed a nitrogen-free diet. This assay is commonly conducted with cecectomized roosters to eliminate the confounding effects of microbial fermentation of cecal contents (Parsons, 1986). There are several advantages of this assay:

1) Roosters do not need to be sacrificed to collect excreta as this assay is based on the total tract digestibility.

2) Only a very small amounts of test ingredient are needed (e.g. 40 g)

3) Precision feeding eliminates the need to use a marker,

4) Forced feeding allows the consumption of the whole quantity of the test ingredient to regardless of the palatability or particle selection.

5) This assay saves money and time (no need to grow a flock of broiler chicks)

There are also several disadvantages related to the rooster assay:

1) Fasting roosters and feeding them through a tube do not represent the normal feeding behavior.
2) Fasting will result in a negative nitrogen balance (not in physiological state) due to the increased protein catabolism leading to increased uric acid excretion (may lead to errors in estimating the endogenous losses).
3) Feeding only the test ingredient without providing other ingredients (e.g. fat) may affect the digestion process.
4) Feces are contaminated with uric acid and may be other materials like feathers or foreign materials. Contamination with uric acid can be a problem when feeding overheated proteins leading to excretion of AAs in the urine as metabolites (Ravindran and Bryden, 1999).
5) Some undigested proteins may be fermented in the large intestine of the birds (Bryden et al., 1990).
6) Results obtained from the rooster assay may not be applied for young birds for some ingredients (Ravindran and Bryden, 1999; Garcia et al., 2007; Adedokun et al., 2014).

Chick assays involve *ad libitum* feeding of growing chickens the test ingredient in a semi-purified diet (test ingredient is the only source of AAs) for a period of time then the birds are sacrificed to collect ileal contents for AA analysis. The AA contents of the ilium are related to the concentration of AAs fed to calculate AA digestibility coefficients. The chick assays can be classified into three assays based on correction for the endogenous losses: Apparent Ileal Digestibility (AID), True Ileal Digestibility (TID) and Standardized Ileal Digestibility (SID)

The AID is defined as the disappearance of the ingested AAs from the gastrointestinal tract between proximal ileum to the distal ileum (Stein et al., 2007). This method does not take into account any correction for the endogenous losses (for example: sloughed intestinal cells, enzymes, mucous, bacteria… etc.). As a result, the digestibility coefficients for the AA may be underestimated). The AID can be calculated as follow:

$$AID\ (\%) = [(AA_{Intake} - Ileal\ AA_{Outflow}) / AA_{Intake}] \times 100 \text{ (without a marker)}$$

$$AID = 100 – [100 \times (Marker_{Feed\ (\%)} / Marker_{Digesta\ (\%)}) \times (AA_{Digesta\ (\%)} / AA_{Feed\ (\%)})] \text{ (With a marker)}$$

The TID is corrected for the endogenous losses (both basal and specific). The basal losses are related to the physical flow of the digesta in the gut and are independent of the diet composition while the specific losses are dependent of the diet (Stein et al., 2007). The correction for the endogenous losses can be achieved by comparing the fed birds (test birds) to birds fed a protein free diet. The SID accounts for only the basal endogenous losses which can be measured using a protein free diet or regression (Furuya and Kaji, 1989, Lemme et al., 2004; Adedokun et al., 2014).

The advantages of chick assays are:

1) Chicks are fully fed (in normal physiological state).
2) No microbial fermentation for the undigested AAs.
3) Corrected for endogenous losses. (i.e. TID and SID)
4) Digestibility are coefficients obtained from growing chickens
5) NO AAs of urine origin

The disadvantages of chick assays are:

1) Chicks need to be sacrificed.

2) The AID Can be influenced by feed intake and dietary protein (Lemme et al., 2004).

There has been a large volume of research published in the past three decades on the apparent (Ravindran et al., 1998; Ravindran et al., 1999; Huang et al., 2005; Huang et al., 2006), true (Angkanaporn et al.,1996; Rutherfurd et al., 2004; Rutherfurd, et al., 2007; Kong and Adeola, 2010) and standardized (Adedokun et al., 2007; Bandegan et al., 2009; Bandegan et al., 2010; ozłowski et al., 2012) ileal AA digestibility of various feedstuffs. In addition, data on AA digestibility using rooster assay are well documented (Fernandez and Parsons, 1996; Fastinger et al., 2006; Gao et al., 2012; Loeffler et al., 2013). Recently, commercial databases compiling data on digestible AAs for poultry are available for nutritionists and feed formulators. Two common AA databases are Ajinomoto Heartland Inc. (AHI; Chicago, IL) and Evonik Industries (ED; Burr Ridge, IL). The AHI database provides digestible AAs data obtained from the rooster assay while the ED database data are based on the SID of the chick assay.

Nutrient Variability of Feed Ingredients

One important characteristic of nutrient content of a feed ingredient is the inherent nutrient variability. The nutrient content in subsequent batches of the same feed ingredient coming to a feed mill for instance will never be exact. Several factors can contribute to the variation in nutrient content including genetic background of the plant, agricultural conditions where the plant is grown (e.g. fertilization rates), stressors (e.g. drought, extreme heat early frosts and diseases) and processing conditions (e.g. mechanical extraction or chemical extraction). Other factors like sampling and laboratory analysis could add up to the nutrient variability (Cromwell et al., 1999; Cromwell et al., 2000). Table 2.1 shows the extent of crude protein variation in different countries. Crude protein in corn samples obtained from France and Italy is less variable than samples obtained from countries like Austria Brazil and Russia. Bakery meal, fish meal, and poultry byproduct meal for instance are characterized with high variability (Table

2.2) compared to potato protein or soybean meal due to the raw materials used in the production of these ingredients. The nutrient variability must be understood and controlled in order to formulate feeds that meet the nutritional requirements of poultry. Ignoring nutrient variability could adversely affect the performance of the birds as the nutrient requirements are not fully met. Lerman and Bie (1975) discussed the problem of batch-to-batch variation in nutrient contents as one of two potential obstacles to maximize profitability. The problem of nutrient variability in feedstuffs was recognized in the 1960s (Deyoe, 1964). Since then several solutions have been proposed to overcome nutrient variability. Chung and Pfost (1964) suggested sampling and assaying all batches of incoming ingredients and then separating the batches based on the average nutrient content into "above average" and "below average" batches as a way to reduce nutrient variability. Nott and Combs (1967) suggested including a safety margin by subtracting one-half standard deviation from nutrient contents of feedstuffs as a method to account for nutrient variability. Rahman and Bender (1971) recommended using stochastic programming in feed formulation models. Shutze and Benoff (1981) suggested statistical procedures to deal with the nutrient variability.

Maximum Inclusion Level of Feed Ingredient

Feedstuffs vary in the quality of nutrients they contain and such a variation can impact the performance of the birds. The nutrient quality may be indicated by the presence of non-nutritive compounds like fiber, anti-nutritional factors, or AA profile, etc. which can produce toxic-like effects in some cases. Toxicity, as defined by NRC (1994), is any adverse effect on bird performance. Some ingredients like corn have good nutrient quality and as a result they can be included in poultry feeds at high levels without problems. Other ingredients can only be included in the feeds at certain levels because of the reduced nutrient quality. Raw soybeans contain trypsin inhibitor that interferes with protein digestion if not processed properly (Borchers et al., 1948; Han et al., 1991; Herkelman et al., 1993). Other antinutritional substances like raffinose, and stachyose also present in soybeans and can reduce digestibility for poultry (Coon et al., 1990; Parsons et al., 2000). The presence of gossypol pigments, cyclopropenoid fatty acids, high fibre and poor protein quality may limit the use of cottonseed meal in poultry diets (Nagalakshmi, 2007). Feeding high levels of cottonseed meal has been reported to cause egg discoloration and growth depression (West, 1955; Heywang et al., 1965; El Boushy and Raterink, 1989). The high glucosinolate content in certain rapeseed cultivars were found to produce toxic effects in poultry (Summers et al., 1971; Smith et al., 1976; Kloss et al., 1994). Ingredients like some cultivars of grain sorghum with high levels of tannins and wheat with high content of non-starch polysaccharides (**NSP**) were reported to produce inferior performance (Chang et al., 1964; Del Alamo et al., 2008; Torres et al., 2013; Kiarie et al., 2014). High fiber ingredients (e.g. DDGS, safflower seed meal, sunflower meal) could lead to reduced performance especially for young birds. Similarly, poor AA. profile of some ingredients can lead to similar effects if not supplemented with synthetic AAs. Feeding any ingredient having poor quality nutrients or antinutritional factors can adversely impact the performance when the level of the ingredient is high enough for a nutrient to become limiting or an antinutritional substance to reach the toxicity threshold. In fact, the maximum safe level (**MSL**) of the ingredient has to be estimated accurately to avoid any bad consequences. Experimentally, to estimate the MSL of such ingredient, increasing levels of the ingredient has to be fed to groups of birds and the biological response (e.g. BW gain, feed intake, etc.) is eventually measured. Typically, a multiple range test is used to define the MSL statistically. Duncan's multiple range test was used extensively in nutrition literature to determine the MSL of test ingredients for poultry (Leeson et al., 1987; Jankowski et al., 2003; Lee et al., 2009; Sun et al., 2013). Fisher's LSD test (Tsiagbe et al., 1987; Castanon et al., 1990; Persia et al., 2003; Khempaka et al., 2009) and Tukey's multiple range test (Corazza and Saylor, 1983; Hidalgo et al., 2004; Jacop and Carter, 2008; Lokaewmanee et al., 2012) were also used to define the MSL but to a lesser degree compared to Duncan's test. Other tests like Dunnett's test (Ameenuddin et al., 1983; Shires et al., 1983), Scheffé's multiple comparison test (Baurhoo et al., 2011), Student Newman Keuls multiple range test (Blair et al., 1986; Mikulski et al., 2012), Ryan-Einot-Gabriel-Welch multiple range test (Hetland and Svihus 2001; Øverland et al., 2010) and Bonferroni's post priori test (Olver, 1998) were rarely used. The response to the test ingredient could be tested for any potential trend (e.g. linear, quadratic, etc.) by regression analysis. Several reports employed regression analysis to examine whether or not there is a trend in the response (e.g. linear and quadratic effects) due to feeding increasing levels of the test ingredient (Proudfoot and Hulan, 1988; Loar et al., 2010; Mclea et al., 2011; Gopinger et al. 2014). From a statistical stand point, estimating the MSL by a multiple-range test is inappropriate for several reasons (Petersen, 1977; Dawkins, 1983; Lowry; 1992; Pesti et al., 2009). First, the levels of the test ingredient, which is continuous variable, are assumed to be discrete by the multiple range procedure. Second, this procedure distinguishes between levels of test ingredient (no exact estimate). lastly, not all tests have the same power in detecting differences; more conservative tests (Scheffe, 1953) can result in fewer differences and less conservative tests (e.g. Fisher's LSD test, 1935) can result in false differences.

Nutritional Requirements

One important step during feed formulation is the knowledge of nutritional requirements of the birds. The nutritional requirements vary depending several factors such as the age and the type of the bird. Poultry requires many key nutrients that should be accounted for during feed formulation to maximize productivity and prevent any deficiency symptoms. There are several sources for nutritional requirements available to nutritionists like NRC (NRC, 1994), British ARC, and commercial companies such as Cobb Vantress (Cobb-Vantress Inc., Siloam Springs, AR) and Aviagen (Aviagen Inc., Huntsville, AL). These sources provide nutritional requirement data for energy, protein and amino acids, minerals, and vitamins. Protein and amino acids are perhaps the most important nutrients in poultry nutrient that received much attention over that past few decades. Economic, environmental and nutritional factors have shed light on the importance of these nutrients.

Protein and Amino Acid Requirements

Proteins are very complex macromolecules composed of chains of amino acid resides varying in size and composition. It is generally accepted among nutritionist that there is no bird requirement for protein *per se* and the requirements are for the amino acids. For maximum body protein synthesis, the requirements of about 10 essential amino acids (**EAAs**) plus some other quantity of nonessential amino acids (**NEAAs**) have to be met. The commercial availability of many synthetic AAs has led to decreasing crude protein (**CP**) levels when formulating poultry diets to reduce feed costs and environmental pollution due to nitrogen wastes (e.g., more corn less soybean in the diet). Feeding diets low in CP levels while meeting the requirements of EAAs only has been shown to modify carcass composition and reduce performance for broiler chickens (Alleman et al., 2000; Sklan and Plavnik, 2002; Corzo et al., 2005; Dean et al., 2006). Addition of NEAAs to the low CP diets as a source of nitrogen to synthesize the other NEAAs resulted in improvements in performance. Therefore, the reduction in performance seen when feeding the low CP diets was attributed to insufficient NEAA content. This clearly shows that there is a requirement for some quantity of NEAAs or at least amino nitrogen. The amino nitrogen source could be NEAAs or excesses of the EAAs (Heger, 2003). The NEAAs requirements seem to be an oxymoron since poultry diets are formulated without considering their contents of NEAAs while they are building blocks of body proteins. Historically, there have been several attempts to account for the NEAA requirements or amino nitrogen during feed formulation. Heger (2003) detailed various methods used to define the optimal EAAs to NAAs ratio and discussed some issues associated with these methods.

Methods Used to Account for NEAA or Amino Nitrogen Requirements

Many ways have been proposed to account for the NEAA or amino nitrogen requirements during feed formulation. Young and Zamora (1968) examined the quantitative relationship between EAAs and NEAAs in weanling rats by varying the proportion of total EAAs and total NEAAs of diets supplying either 2.23 or 2.90% dietary nitrogen. They reported that the maximum growth rate was reached when the ratio of total EAAs per gram of total dietary nitrogen was between 3.37 to 4.71 for the diets containing 2.23% dietary nitrogen and 3.03 and 4.04 for the 2.90% nitrogen diets. Ikemoto et al. (1989) investigated the optimal ratios of EAAs to total AAs in rats. Lenis et al. (1999) studied three EAA nitrogen to NEAA nitrogen ratios (38:62, 50:50, and 62:38) at three total nitrogen levels (18.8, 22.9, and 30.0 g/kg) in growing pigs. They concluded that the optimal EAA nitrogen: NEAA nitrogen ratio for maximum nitrogen retention was 50:50 and the ratios are more important at lower dietary protein levels. Heger (2003) recalculated the ratio of EAA nitrogen to total AA nitrogen (E:T) for literature data on rats, pigs and poultry. He concluded that the optimum E:T ratios for growth or protein deposition do not differ considerably between species and depend on dietary nitrogen levels (E:T ratios are lower at higher dietary nitrogen and *vice versa*). Theoretically, estimating the ratio of an EAA (e.g. lysine) to protein in feeds could account for the NEAA requirements during feed formulation.

Protein in Feedstuffs

The CP content of feed is calculated by multiplying the nitrogen content of a feed sample by a conversion factor of 6.25. The nitrogen content of feed samples can be determined by proximate analysis system (e.g. kjeldahl or nitrogen analyzer). The conversion factor of 6.25 was proposed in the early 1900s after recognizing that some proteins mostly of animal origin such as serum albumin, serum globulin from blood and casein from milk contained 16% nitrogen (Jones, 1931). The conversion factor 6.25 used in estimating protein level assumes all proteins contain 16% nitrogen. In fact, not all proteins in nature contain 16% nitrogen as they can vary considerably in nitrogen contents. In addition, a portion of the estimated CP is originated from non-protein nitrogen compounds (e.g. nucleic acids, amino sugars, creatine, etc.) which are not related to proteins and may not be utilized very well in the biosynthesis of NEAAs (Heger, 2003). Therefore, the only representative of AAs in feedstuffs is true (net) protein. The true protein level of a feed ingredient can be estimated by multiplying the nitrogen contents of that ingredient by specific N:P conversion factor (Mosse, 1990). The specific N:P conversion factor or K can be calculated by averaging two N:P conversion factors: K_A (the ratio of the weight of the anhydrous AA residues to N content from AA residues; Mosse et al.,1985) and K_P (the ratio of the weight of the anhydrous AA residues to total N content; Sosulski and Holt, 1980).

The Specific N:P Conversion Factors of Feedstuffs

The indiscriminate use of the conversion factor 6.25 in the early 1990s led several investigators to isolate proteins from various foodstuffs and calculate specific N:P conversion factors (Jones, 1931). The new conversion factors were thought to yield more accurate estimates of true protein than the 6.25 but there was a limitation in the accuracy due to not considering the non-protein nitrogen compounds in the calculations. Tkachuk (1969) corrected the calculation error in the calculations by taking into account the nitrogen from non-protein nitrogen compounds when he estimated specific N:P conversion factors for several cereals and oil seeds (Table 2.3). Sosulski and Imafidon (1990) reported conversion factors for 23 primary food products (Table 2.3) with an average of 5.68 ± 0.30 and suggested a common conversion factor of 5.70. Mosse (1990) calculated K_A and K_P values for various foodstuffs (Table 2.3) and suggested the average of the two values (K) as an approximation of the true protein content. Mariotti et al. (2008) proposed a default conversion factor of 5.60 based on data compiled from literature. Sriperm et al. (2011) determined the factors K_A, K_P and K for five common feed ingredients commonly used in poultry feeds and observed that the factor K_A gave closest estimate of true protein than the factor K (Table 2.3).

Estimation of Nutritional Requirements

Broken line or spline models are the most commonly applied non-linear regression models to estimate the nutritional requirements in poultry and other species. Broken line models are based on the assumption that feeding a particular nutrient (e.g. lysine) in increasing concentrations will produce a change in the biological response up to some point (requirement) where the response plateaus thereafter. The change in response could be an increase for some parameters like growth or a decrease for others (e.g. feed conversion ratio). The ascending (or descending) part or the response can be considered a straight line as with the broken-line linear model (**BLL**) or a smooth curve as in the broken-line quadratic model (**BLQ**). The nutritional requirement is defined here as the nutrient level that maximizes the response and the requirement is estimated at the breakpoint of the ascending line and plateau. The estimate of the requirement is usually followed by an estimate of the standard error indicating how good an experiment is (e.g. sufficient replication number). The coefficient of determination (R^2) gives important information on the goodness of model fit.

The BLL model (Robbins et al., 1977; Robbins et al., 1979) is defined mathematically by:

$$y = \begin{cases} Maximum\ Response, & if\ x > Requirment \\ Maximum\ Response\ +\ Rate\ Constant\ \times\ (Requirment - x) & if x \leq Requirment \end{cases}$$

where y is the response variable and x is the nutrient level

The BLL fits two linear segments (i.e. the ascending or descending and the plateau) of the nutritional response. The BLQ model (Vedenov and Pesti, 2008) can be defined mathematically by:

$$y = \begin{cases} Maximum\ Response, & if x >\ Requirment \\ Maximum\ Response\ +\ Rate\ Constant\ \times\ (Requirment\ -\ x)^2 & if x \leq\ Requirment \end{cases}$$

where y is the response variable and x is the nutrient level

The BLQ fits a quadratic segment (ascending or descending) and a linear segment (plateau) of the nutritional response. Like the BLL the BLQ does not take into account the toxicity level due to high nutrient intake. The BLQ is usually more difficult to fit and produces a wider confidence interval compared to the BLL (Pesti et al., 2009).

The broken-line methodology has been used extensively to estimate the requirements of many nutrients like amino acids (Baker et al., 1996; Webel et al., 1996; Labadan et al., 2001; Dozier et al., 2009), vitamins (Chung and Baker, 1990; Edwards et el., 2002; Wen et al., 2014) and minerals (Aoyagi et al., 1995; Oviedo-Rondon et al., 2001; Dhandu et al., 2003; Driver et al., 2005).

Table 2.1. Descriptive statistics of crude protein for corn samples collected between 2005 – 2010 from different origins (AminoDat 4.0; Evonik Degussa, 2010).

Origin	Sample size	Mean	CV [1]	Minimum	Maximum
Austria	10	8.1	14.8	6.5	10.0
Brasil	108	8.3	13.4	6.2	11.0
France	15	7.6	5.4	7.1	8.4
India	12	8.4	10.1	6.4	9.7
Indonesia	14	8.7	8.3	7.8	10.0
Italy	31	7.8	4.5	7.2	8.7
Poland	18	9.2	8.3	7.9	10.5
Russia	11	8.5	13.4	7.1	10.9
USA	334	7.8	8.8	5.7	10.2

[1]Coefficient of variation

Table 2.2. Descriptive statistics of crude protein for samples of different ingredients collected between 2005 – 2010 (AminoDat 4.0; Evonik Degussa, 2010).

Ingredient	Sample size	Mean	CV [1]	Minimum	Maximum
Bakery Meal	409	10.8	16.4	4.4	15.5
Fish Meal	811	59.2	15.8	29.9	74.6
Meat and Bone Meal	314	44.8	16.8	21.7	65.2
Potato Protein	62	75.3	2.3	70.0	79.0
Poultry Byproduct Meal	659	56.5	14.1	31.4	71.5
Soybean Meal	1218	46.9	4.2	36.8	52.4
Wheat	733	11.9	14.4	7.7	17.7

[1]Coefficient of variation

Table 2.3. Specific N: P conversion factors (K_A and K_P) of various feedstuffs obtained from the world's literature [1].

Ingredient	Tkachuk, 1969		Moss, 1990		Sosulski & Imafidon, 1990		Sriperm et al., 2011	
	K_A	K_P	K_A	K_P	K_A	K_P	K_A	K_P
Barley	5.67	-	5.72	5.26	-	-	-	-
Casein	-	-	-	-	6.15	-	-	-
Corn	-	-	-	-	5.72	-	5.68	5.06
Corn DDGS	-	-	-	-	-	-	5.74	4.99
Fish	-	-	-	-	5.82	-	-	-
Flax meal	5.41	-	-	-	-	-	-	-
Lupin	-	-	5.46	5.27	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	5.37	4.77
Milk		-	-	-	6.02	-	-	-
Millet	5.68	-	5.53	5.17	-	-	-	-
Oats	5.5	-	5.52	5.31	-	-	-	-
Pea	-	-	5.48	5.25	5.4	-	-	-
Poultry by-product	-	-	-	-	-	-	5.45	4.81
Rapessed meal	5.53	-	-	-	-	-	-	-
Rye	5.64	-	5.58	5.12	-	-	-	-
Sorghum	-	-	-	-	5.93	-	-	-
Soybean meal	5.69	-	5.67	5.38	-	-	5.64	5.13
Sunflower	5.36	-	-	-	-	-	-	-
Triticale	5.76	-	5.61	5.24	-	-	-	-
Wheat	5.61	-	5.56	5.36	5.75	-	-	-
Wheat bran	5.26	-	-	-	-	-	-	-

[1] K_A is the ratio of the weight of the anhydrous AA residues to N content from AA residues and K_P is the ratio of the weight of the anhydrous AA residues to total N content.

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CHAPTER 3

REDUCING CRUDE PROTEIN VARIABILITY AND MAXIMIZING SAVINGS WHEN FORMULATING CORN- SOYBEAN MEAL BASED FEEDS [1]

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SUMMARY

Crude protein in corn and soybean meal have been documented to vary and such inherent variability can result in under- or over-feeding of crude protein when feeds are formulated, leading to reduced bird growth, added input costs, and increased environmental pollution. The purpose of this study was to compare two grain handling techniques and two feed formulation methods (linear versus stochastic programming) to reduce crude protein variability in finished feeds and determine resulting costs or savings. The two grain handling techniques were placing all the random batches of each delivered ingredient in to 1) a single bin (1-bin method), or 2) segregating above and below average samples into two bins (2-bin method). A fast way of estimating the composition of the ingredients is now available (NIRS). Microsoft Excel workbooks were constructed to solve broiler starter feed formulation problems. Formulating feeds by linear and stochastic models based on the 2-bin method reduced crude protein variability by at least 50% compared to the 1-bin method. Formula cost was reduced by ~ 20 cents per ton (averages of August 2012 USA ingredient prices) when the 2-bin method was used with the linear model. Formulating feed with a margin of safety increased formula cost by $3.40 per ton. Stochastic feed formulation increased formula cost to meet the specified crude protein level in feed at any probability of success and formula cost was reduced substantially with the 2-bin method (up to $6.47 per ton). The magnitude of savings and reduced feed variability suggested that regardless of the costs associated with building extra bins, the 2-bin method can be economically efficient in the long run. Therefore, it could be possible to split the batches of feed ingredients at a feed mill into above- or below-average bins prior to feed formulation to reduce crude protein variability and to maximize savings.

Key words: Nutrient variation, Crude protein, Linear programming, Stochastic programming

DESCRIPTION OF PROBLEM

Corn and soybean meal (**SBM**) are the most commonly used feed ingredients by the poultry industry and are the major sources of protein in corn-SBM based feeds. Crude protein (**CP**) levels in corn and SBM have been reported to vary due to several factors such as genetic background and agricultural conditions. Practical feeds are usually formulated by linear programing software based on the expected CP means of corn and SBM which can be obtained from ingredient composition tables. Formulating such feeds based on the expected CP means, but not the actual means, might reduce the performance of the birds as the chance of achieving the CP limit in the feed is 50% of the time (half of the samples will be below average). Furthermore, CP variability can increase input costs and environmental pollution with the over-fed nitrogen in the 50% of samples above the required minimum.

This problem was recognized in the 1960s and since then there have been few changes in the methodology to control nutrient variability in feedstuffs. Nott and Combs [1] recommended adding a margin of safety to account for CP variation in feedstuffs by subtracting one half standard deviation from CP means. Rahman and Bender [2] suggested the use of non-linear "stochastic" programming that takes nutrient variability into account when formulating feeds. Chung and Pfost [3] suggested separating the batches of feed ingredients based on the average nutrient content into above-average or below-average batches as a way to reduce nutrient variability. "Batches" represents some unit of ingredients with a known ingredient composition, in this case it could represent carloads of an ingredient delivered to a feed mill. The objectives of this study were to build on the concept of batch separation [3] and to evaluate the effects of separation on 1) CP variability of finished broiler feeds formulated by linear and stochastic models; and 2) formula cost of feeds formulated by a linear model or a stochastic model at different probabilities of success.

MATERIALS AND METHODS

Data Source

The descriptive statistics of CP for corn and SBM used herein were obtained from a previous work performed at the Department of Poultry Science, University of Georgia [5]. Corn and SBM samples in this work were collected from various regions of the United States and Canada during 2009. These samples were analyzed in duplicate by the Dumas combustion procedure [6], to estimate CP content (Table 3.1).

Workbook Construction

CP distributions for corn and SBM in Figures 3.1 and 3.2 could not be proven to be non-normal. Thousands of simulated CP values were generated in Microsoft Excel to estimate measures of variation of CP in finished feed samples or batches. The CP mean for above and below average samples (truncated distributions) was estimated from simulations (Figures 3.3 and 3.4). Four Microsoft Excel workbooks were constructed: Workbooks 1 and 2 (Figures 3.5 & 3.6) contain 10,000 and 2,500 simulated batches of feed, respectively, and were designed to calculate: 1) measures of variation of CP in the finished feed (coefficient of variation (**CV**) and standard deviation (**SD**)), and 2) proportion of batches of feed below or above specified CP levels. Each batch of feed in Workbook 1 was formulated by the linear feed formulation method, WUFFFDA [7], from unseparated batches of corn and SBM (1-bin method) using the population means for corn (CP= 6.90%) and SBM (CP= 47.51%). Batches of feed in Workbook 2 were formulated from a super-set of Workbook 1 from separated batches of corn and SBM based on the population mean of CP for each ingredient. The batches of corn and SBM were separated into below- or above-average bins (2-bin method) and the mean for each bin/truncated distribution (obtained from simulation) was used for feed formulation. The ingredients that constituted each batch of feed were corn, SBM, poultry fat, limestone, dicalcium phosphate, common salt, mineral premix, vitamin premix, and Dl- Methionine. Workbooks 3 and 4 (Figures 3.7 and 3.8) were constructed according to Pesti and Seila [8] as simple stochastic workbooks to formulate feeds with both formulation methods (1-bin and 2-bin) at different probabilities of success. Feed was formulated with a margin of safety (Table 3.2) by subtracting one-half standard deviation from the average CP values for corn and SBM as suggested by Nott and Combs [1]. All feeds discussed herein were formulated to meet the nutritional requirements of starting broilers [9] with average USA prices for August 2012 [10]. A detailed description of how these workbooks are constructed is discussed elsewhere (Appendix A) [11].

RESULTS AND DISCUSSION

CP Means for the Truncated Distributions

The normal distribution simulations in Excel estimated means of 6.41% CP in below-average corn, 7.36% CP in above-average corn, 46.40% CP in below-average SBM and 48.58% CP in above-average SBM. Chung and Pfost [3] suggested using formulas to calculate the means for the truncated distributions. Their formula for above-average batches is $\vec{X} = m + \frac{2}{\sqrt{\pi}} * S$ while the formula for below-average batches is $\vec{X} = m - \frac{2}{\sqrt{\pi}} * S$ (where m is the mean of CP and S is the standard deviation of CP). Applying these formulas to our example would result in CP means of

6.23, 7.57, 45.91, and 49.11% for below-average corn CP, above-average corn CP, below-average SBM CP, and above-average SBM CP, respectively. Assuming that the Excel function with 1,000 simulations is correct, the Chung and Pfost formula underestimates the below-average means by 1-3% and overestimates the above-average means by 1-3%. Chung and Pfost [3] assumed normality for CP in their example and this assumption could lead to over or under estimation of CP means, resulting in deficient or excessive CP content in the finished feed [12].

Measures of CP Variation

CP variability can be reduced when corn and SBM batches are separated as in the 2-bin method (Table 3.3). The CV was reduced approximately 57% (1.20 vs. 2.81) when the batches of these ingredients were separated. As the standard deviation decreased from 0.65 to 0.28 with batch separation, the CP values from both directions (below and above 23%) of the normal distribution curve are brought closer to the CP mean, resulting in a tall and narrow frequency distribution (Figure 3.9). Grouping the whole CP populations of corn and SBM that are characterized with great dispersion into smaller and more uniform sub-populations that are less dispersed resulted in the reduction in the CP standard deviation. The reduction in CP variability is important in practical situations as poultry producers would like to guarantee that the diet is less variable in CP and delivers the specified CP level most of the time. Although none of the two formulation methods increased the batches of feeds having at least 23% (which is the desired CP level in this study) the 2-bin method resulted in a large number of feed batches (96%) above 22.5% and a smaller number of batches (3.35%) above 23.5% compared to 78% and 21.94% for the 1-bin method, respectively. In other words, 93% of the batches of feeds formulated by the 2-bin method are expected to have a CP in the range 23 ± 0.5% compared to only 56% for the 1-bin method. Maintaining CP levels in a narrow range around the specified CP level can minimize the impact of CP variability on the performance of the birds as the chance of getting low CP levels (e.g. below 22.5%) is reduced. Furthermore, feeding excessive CP (e.g. above 23.5%) can be avoided by formulating feeds with this method, thus minimizing the impact of excessive nitrogen excretion on the environment. Batch separation has the same effects on CP variability when stochastic programming is used at any probability of success (Table 3.4). As the probability of success increases, CP content increases accordingly to achieve the level of assurance with the 2-bin method requiring less average CP to achieve the level of assurance. This is important to minimize formula cost and excessive nitrogen with stochastic feed formulation. Figures 3.10 and 3.11 show that formulating feeds by stochastic programming at 80% probability of success shifts the distribution of CP to the right, with the 2-bin method producing tall and narrow frequency distribution compared to the 1-bin method. This is true when formulating feeds by stochastic programming at any probability of success greater than 50%. The emphasis of the current study was on CP (not amino acids) since protein is an important nutrient to consider in quality control of raw materials. The reduction in CP variability with the 2-bin method was a result of reduced variability of its components, amino acids and non-protein containing compounds. CP of any feed ingredient can be considered as a function of its components, and conversely. Any changes in the values of CP influence the constituent amino acids. Digestible amino acids are a linear function of CP. Plants contain the same proteins with constant amino acid ratios even when they contain different amounts of CP. Therefore, batch separation reduced individual amino acids' variability (total or digestible) in the finished batch of feed.

Economic Comparisons for Feed Formulation Methods

In this study, formulating broiler feed by a linear programming model and the 2-bin method resulted in a ~ 20 cents per ton reduction in formula cost compared to the linear feed formulation with the 1-bin method (Table 3.2). The reduction in formula cost was due mainly to amino acid usage since the CP in amino acids is without variation. The feed formulated using the 2-bin method was slightly higher in corn (47.34 vs. 47.33%) and slightly lower in SBM (41.25 vs. 41.24%). These marginal differences accounted for the observed difference in formula cost. Formulating feed with an added margin of safety as suggested by Nott and Combs [1] would increase the formula cost by ~ $3.40 per ton (Table 3.2) without indicating the proportion of feeds meeting the specified minimum (> 80, < 90% from Table 3.5). On the other hand, formulating the same feed using a stochastic programming model at a probability of success greater than 50% can increase formula cost to achieve the assurance level of CP. Increased formula cost for feeds formulated with the margin of safety or stochastic approaches compared to linear feed formulation has been reported and the results for the 1 bin method in Table 3.5 are very similar [13]. Feed formulation using the 2-bin method costs more at low probabilities of success (P < 50%) and less at high probabilities of success (P > 50%) compared to feed formulation using the 1-bin method (Table 3.5). For example, at P = 10% formula cost increased by $3.04 per ton but at P= 90% formula cost decreased by $3.44 per ton for the 2-bin method compared to the 1-bin method. In practice, no one should formulate feed at a low probability of success. Therefore, the 2-bin method can reduce costs if the goal is to formulate feed with a stochastic model. Formula cost increases as a function of CP which is the most important determinant of formula cost besides energy. It might be worthwhile to feed manufacturers to pay extra money in order to increase the probability of success of having the specified CP level in feed. In practice, feed mills use one bin for corn and another one for SBM. Two extra bins must be built if feed mill managers determine a sizable enough advantage to formulate the feeds based on the 2-bin method. In addition, the feed mill must be equipped with a quick CP testing method such as an NIRS system to determine which load of delivered corn and soybean meal should go to which bin in a matter of minutes. The availability of such a quick testing method makes the 2-bin method possible and practical. Each producer can estimate the money they will have available to build the extra bins and implement the feed separation by multiplying the savings from using the 2-bin method by the tonnage of feed to be mixed. In this analysis we chose only to compare 1 vs. 2 bins for separating ingredients. The 2-bin method reduces costs when a high probability of success in meeting specified minimums is desirable. Logical extensions of this work would be to test the relative values of 3 or 4 bins/ ingredient and the practicality of testing each load of ingredients and custom formulation. Linear least-cost feed formulation models have been very useful in the past. Stochastic models capable of determining the value of decreased variation in feeds and minimizing waste from overfeeding may be the technique of the future.

CONCLUSIONS AND APPLICATIONS

1- Crude protein measures of variation of the finished feed can be estimated accurately and efficiently by generating thousands of CP simulations in Microsoft Excel based on any given CP mean and standard deviation.

2- Crude protein variability of the feed can be reduced at least 50% by separating each of corn and SBM batches based on the average into two bins, then formulating feed from these bins (above- and below-average corn bins, above- and below-average SBM bins).

3- Feed costs can be reduced when feed are formulated with the 2-bin method compared to feed formulation with the 1-bin method and the savings are obvious with stochastic feed formulation.

4- In-line equipment is now available to quickly estimate ingredient compositions and facilitate improved formulation techniques and could prove economical if future grain prices are similar to past ones used in this study.

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Table 3.1. Crude protein statistics for corn and soybean meal samples [5]

CP Statistics[1]	Feed Ingredient	
	Corn	**Soybean meal**
N[2]	132	114
Mean, %	6.90	47.51
Minimum, %	5.34	44.10
Maximum, %	8.30	51.50
Variance, %	0.35	2.01
Standard deviation, %	0.59	1.41
Coefficient of variation, %	8.60	2.99

[1]Data are expressed "as fed" basis
[2]Number of samples

Table 3.2. Composition of broiler starter feeds formulated by different feed formulation approaches

Ingredient, %	Feed Formulation Method		
	1-Bin[1]	**2-Bin** [2]	**Margin of Safety**[3]
Corn	47.33	-	46.03
SBM	41.25	-	42.35
Below-average Corn	-	23.67	-
Above-average Corn	-	23.67	-
Below-average SBM	-	20.62	-
Above-average SBM	-	20.62	-
Poultry Fat	7.31	7.31	7.51
Limestone	1.40	1.40	1.40
Dicalcium Phosphate	1.62	1.62	1.62
Common Salt	0.45	0.45	0.45
Vitamin Premix	0.25	0.25	0.25
Mineral Premix	0.15	0.15	0.15
DL-Methionine	0.24	0.24	0.24
Calculated composition:			
ME (kcal/g)	3.20	3.20	3.20
CP minimum (%)	23.00	23.00	23.00
Formula cost ($/ton)	470.60	470.40	474.00
Savings ($/ton)[4]	0.00	-0.20	3.40

[1]Feed formulated with a linear programming model (WUFFFDA) without batch separation (1-bin method) with 6.9% corn CP and 47.51% SBM CP.
[2]Feed formulated with a linear programming model (WUFFFDA) from separated corn and SBM batches (2-bin method) with 6.41% below-average corn CP, 7.37% above-average corn CP, 46.38% below-average SBM CP and 48.69% above-average SBM CP.
[3]Feed formulated with a linear programming model (WUFFFDA) without batch separation (1-bin method) but with a margin of safety with 6.60% corn CP and 46.80% SBM CP.
[4]Savings calculated by taking the difference between Feed 1formula cost and each of the other feeds.

Table 3.3. Effect of feed formulation method on crude protein variability and the percentage of batches of feed that lies above certain CP levels for feeds formulated by linear programming

	Feed Formulation Method	
CP Statistics	**1-Bin** [1]	**2-Bin** [2]
Mean, %	23.00	23.00
Standard deviation, %	0.65	0.28
Coefficient of variation %	2.81	1.20
CP level (%)	………….. % above CP level…………	
21.0	99.90	100.00
21.5	98.98	100.00
22.0	93.89	99.98
22.5	78.00	96.34
23.0	49.96	49.20
23.5	21.94	3.35
24.0	6.09	0.01
24.5	1.01	0.00
25.0	0.10	0.00
25.5	0.01	0.00

[1]Feed formulation without dividing batches of ingredients
[2]Feed formulation with dividing batches of ingredients into below- or above-average batches

Table 3.4. Effect of feed formulation method on crude protein variability and the percentage of batches of feed that lies above certain CP levels for feeds formulated by stochastic programming

	Probability of Success											
	50%		**60%**		**70%**		**80%**		**90%**		**99%**	
CP Statistics	**1-Bin**	**2-Bin**	**1-Bin**	**2-Bin**	**1-Bin**	**2-Bin**	**1-Bin**	**2-Bin**	**1-Bin**	**2-Bin**	**1-Bin**	**2-Bin**
Mean	23.00	23.00	23.17	23.07	23.35	23.15	23.56	23.24	23.86	23.36	24.61	23.66
SD[1]	0.65	0.28	0.66	0.28	0.65	0.28	0.66	0.28	0.68	0.27	0.69	0.28
CV[2]	2.83	1.23	2.83	1.21	2.78	1.19	2.80	1.21	2.84	1.18	2.82	1.20
CP level (%)	…………………….. % of batches above CP level…………………….											
22.0	93.92	99.98	96.40	99.99	98.07	100.00	99.11	100.00	99.68	100.00	99.99	100.00
22.5	78.22	95.84	83.95	97.79	90.49	98.85	94.47	99.58	97.83	99.90	99.88	100.00
23.0	49.52	49.40	60.07	59.31	69.68	68.34	79.96	78.71	90.13	89.16	99.00	99.02
23.5	21.73	3.16	30.90	5.48	40.99	9.73	53.40	16.83	70.09	29.92	94.59	70.92
24.0	6.11	0.02	10.60	0.03	15.91	0.09	25.55	0.24	41.29	1.22	81.55	11.78
24.5	1.05	0.00	2.03	0.00	3.88	0.00	7.61	0.00	17.67	0.00	56.97	0.17
25.0	0.11	0.00	0.24	0.00	0.64	0.00	1.53	0.00	4.61	0.00	28.96	0.00

[1]Standard deviation
[2]Coefecient of variation

Table 3.5. Probability of success of meeting the specified crude protein level in broiler feeds, feed costs, and the expected savings when feeds are formulated by stochastic programming and two ingredient handling methods

	1-Bin Method[2]		2-Bin Method[3]		
Probability of Success[1] (%)	**Average CP (%)**	**Cost ($/ Ton)**	**Average CP (%)**	**Cost ($/ Ton)**	**Savings ($/ Ton)**
1	21.57	459.21	22.37	464.58	-5.38
5	21.97	461.94	22.55	465.81	-3.87
10	22.19	463.33	22.65	466.48	-3.04
20	22.46	465.28	22.77	467.29	-2.01
30	22.66	466.63	22.86	467.87	-1.24
40	22.84	467.80	22.93	468.38	-0.58
50	23.00	468.91	23.00	468.86	+0.05
60	23.17	470.03	23.07	469.33	+0.70
70	23.35	471.25	23.15	469.85	+1.40
80	23.56	472.70	23.24	470.45	+2.24
90	23.86	474.74	23.36	471.30	+3.44
95	24.12	476.47	23.46	472.00	+4.47
99	24.61	479.82	23.66	473.34	+6.47

[1]Probability of success of meeting the crude protein minimum specification of 23% according to the NRC requirements for broiler starter chickens.
[2]Feed formulated with a stochastic program without batches division with 6.9% corn CP and 47.51% SBM CP.
[3]Feed formulated with a stochastic program from divided corn and SBM batches with 6.40% below-average corn CP, 7.39% above-average corn CP, 46.41% below-average SBM CP and 48.66% above-average SBM CP.

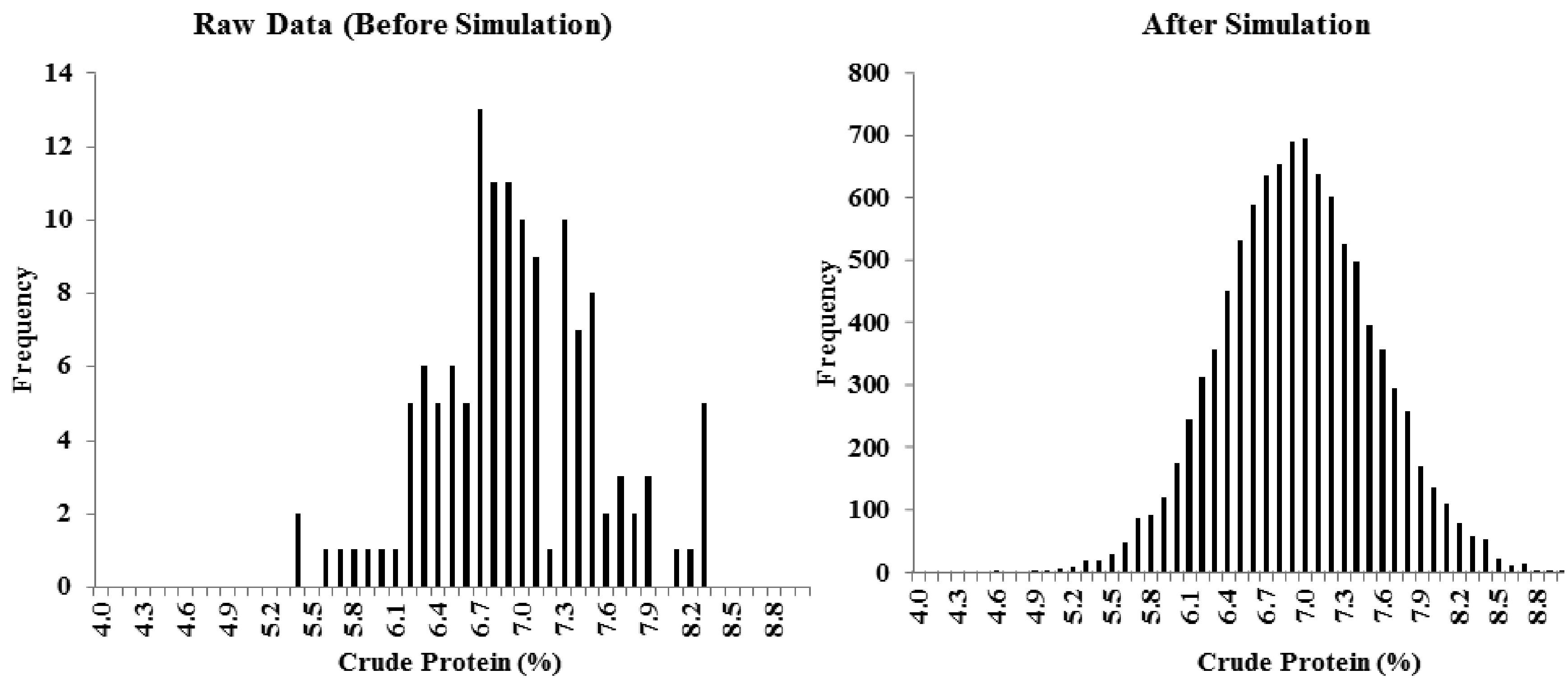

Figure 3.1. Corn crude protein distribution before and after simulation

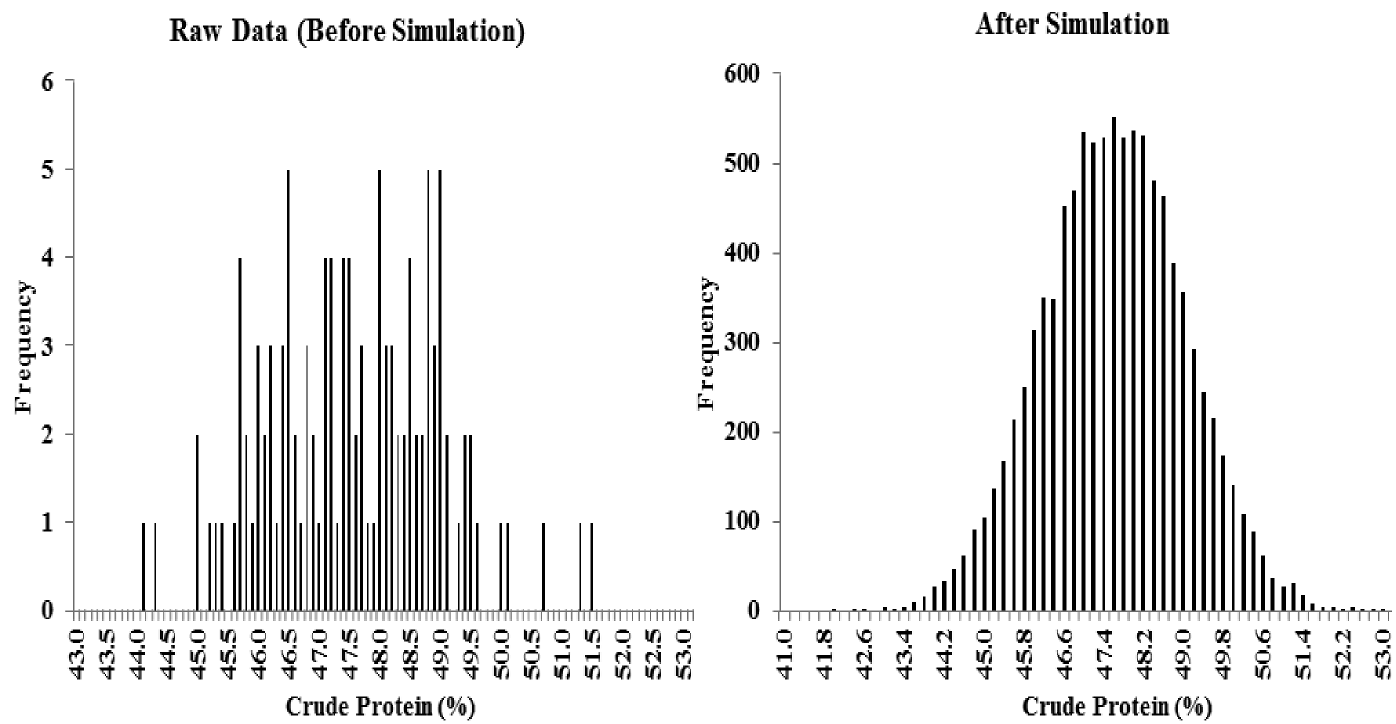

Figure 3.2. Soybean meal crude protein distribution before and after simulation

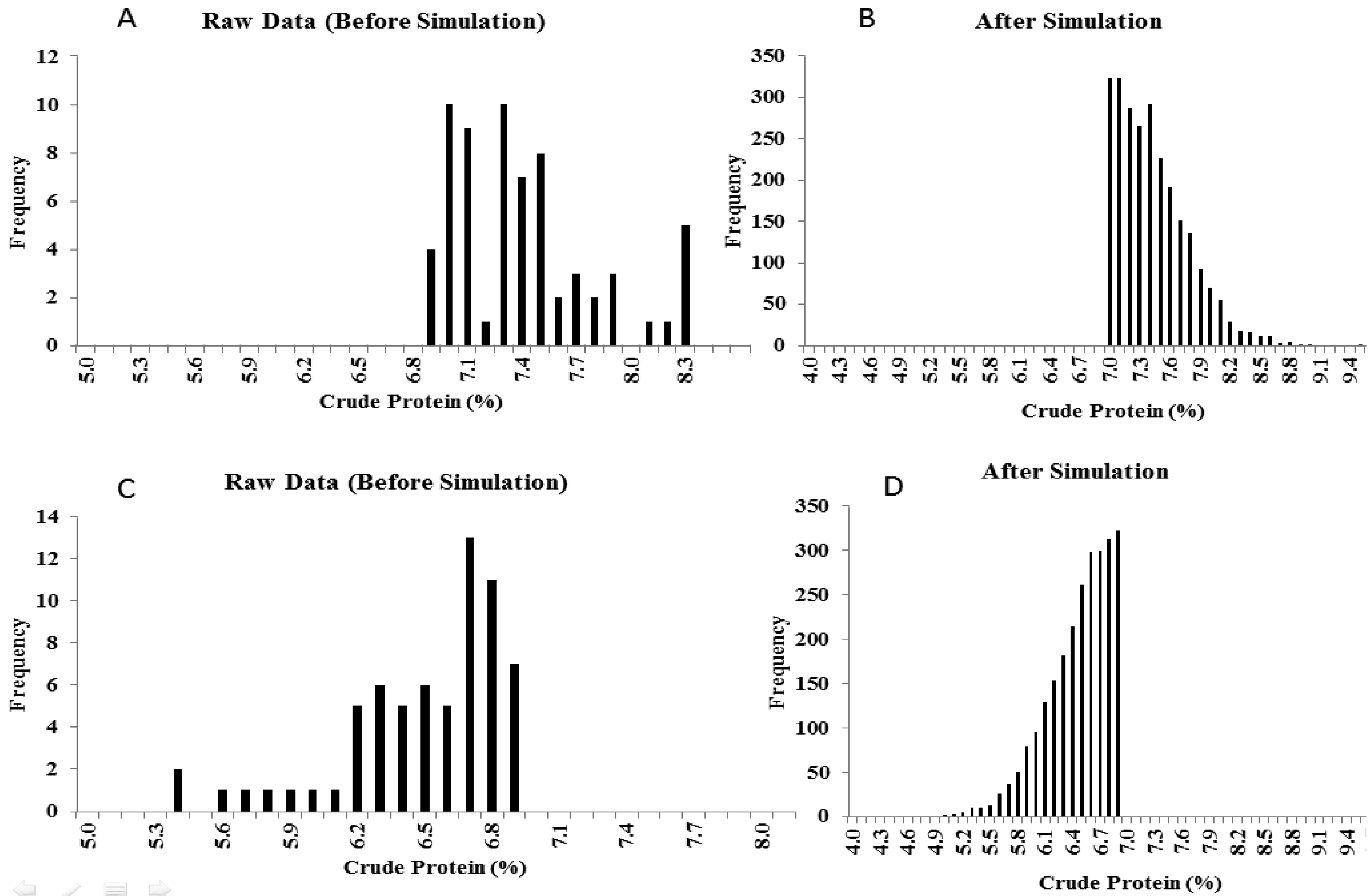

Figure 3.3. (A) Truncated distribution for above-average corn crude protein raw data (before simulation). (B) Truncated distribution for above-average corn crude protein data after simulation. (C)Truncated distribution for below-average corn crude protein raw data (before simulation). (D) Truncated distribution for below-average corn crude protein data after simulation.

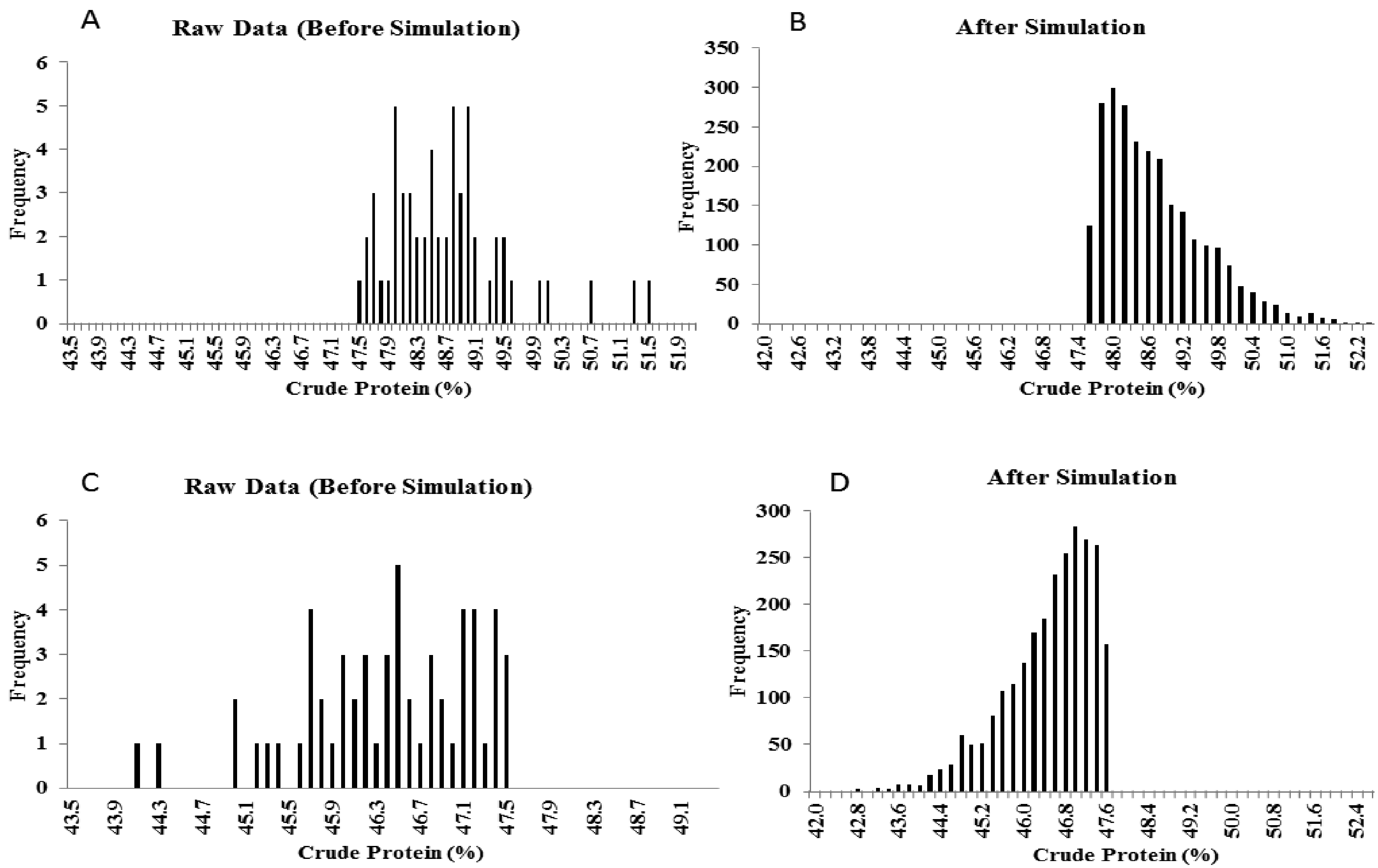

Figure 3.4. (A)Truncated distribution for above-average soybean crude protein raw data (before simulation). (B) Truncated distribution for above-average soybean crude protein data after simulation. (C)Truncated distribution for below-average soybean crude protein raw data (before simulation). (D) Truncated distribution for below-average soybean crude protein data after simulation.

CP Values to be Simulated		
Statistics	Corn	SBM
Mean (%)	6.90	47.51
SD	0.59	1.42
CP Level Tested		23.50

Ingredient Prices $ per 100 lb					
Corn	16.00	DCP	20.00	DL.Met	220.00
SBM	28.00	Salt	2.78		
Poultry Fat	34.00	Vit. mix	370.00		
Limestone	3.00	Min. mix	57.00		

Feed Formulation Results					
Statistics	%			Feeds Mixed	10,000
Mean	23.00	CP Level Tested	23.50	# of Meeting 23%	4,997
SD	0.65	Z-Value	0.77	% of Meeting 23 %	49.97
CV	2.80	% Above Tested Level	21.97	Formula Cost	$23.53

ID	Corn CP (%)	SBM CP (%)	Corn (%)	SBM (%)	Poultry Fat (%)	Limestone (%)	DCP (%)	Salt (%)	Vit. mix (%)	Min. mix (%)	DL-Met (%)	Total (%)	Formula Cost ($)	Diet CP (%)	CP level
1	6.63	46.90	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.62	0
2	7.40	49.21	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.94	1
3	7.72	49.06	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	24.03	1
4	6.16	48.86	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.21	1
5	6.19	47.78	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.78	0
6	6.75	45.34	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.03	0
7	6.81	46.68	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.61	0
8	6.85	47.37	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.92	0
9	7.44	48.38	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.61	1
10	6.08	44.84	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.51	0
11	8.06	48.35	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.90	1
12	7.57	48.41	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.69	1
13	6.95	47.73	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.11	1
14	6.99	47.08	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.87	0
15	6.65	48.37	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.24	1
16	6.93	47.54	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.03	1
17	5.88	47.76	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.62	0
18	6.02	46.57	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.20	0
19	6.24	47.52	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.69	0
20	6.33	44.58	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.52	0
21	6.23	45.58	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.89	0
22	6.49	47.79	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.92	0
23	6.95	47.83	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.15	1
24	6.77	44.81	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.82	0
25	6.58	46.37	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.38	0
26	5.70	46.26	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.92	0
27	6.61	48.88	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.43	1
28	6.57	47.85	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.98	0
29	7.47	45.51	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.45	0

Title | Simulations | G1 | G2 | G3

Figure 3.5. Workbook 1 to estimate crude protein (CP) measures of variation of feeds formulated by the 1-bin method

CP Values to be Simulated		
Statistics	Corn	SBM
Mean (%)	6.90	47.51
SD	0.59	1.42
CP Level Tested	25.00	

Ingredient Prices $ per 100 lb					
Corn	16.00	DCP	20.00	DL.Met	220.00
SBM	28.00	Salt	2.78		
Poultry Fat	34.00	Vit.mix	370.00		
Limestone	3.00	Min.mix	57.00		

Statistics
Mean
SD
CV

	Corn CP (%)	SBM CP (%)	Low Corn CP (%)	High Corn CP (%)	Low SBM CP (%)	High SBM CP (%)	High CP Corn (%)	Low CP Corn (%)	High CP SBM (%)	Low CP SBM (%)	Poultry Fat (%)	Limestone (%)	DCP (%)	Salt (%)	Vit. mix (%)	Mir
10	7.27	47.36	5.58	7.27	47.36	47.98	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
11	5.58	47.31	6.03	7.06	47.31	49.23	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
12	6.03	47.98	6.41	6.90	45.53	48.19	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
13	6.41	45.53	6.60	7.28	44.19	47.75	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
14	6.60	44.19	6.45	7.50	45.68	49.20	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
15	7.06	45.68	5.98	6.92	46.73	48.66	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
16	6.90	46.73	6.80	7.22	47.12	47.60	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
17	7.28	49.23	6.51	7.15	44.12	48.40	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
18	7.50	47.12	6.90	7.02	46.43	47.86	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
19	6.92	44.12	6.80	7.16	46.79	48.08	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
20	6.45	48.19	5.75	7.16	47.41	49.09	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
21	7.22	47.75	6.55	7.44	45.50	48.35	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
22	7.15	46.43	6.40	7.01	47.05	47.68	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
23	5.98	46.79	6.74	7.37	47.18	49.11	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
24	6.80	47.41	6.63	7.58	47.13	47.87	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
25	7.02	45.50	6.85	7.48	47.13	47.61	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
26	7.16	49.20	6.71	8.43	47.00	48.06	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
27	6.51	48.66	6.83	7.85	46.35	48.22	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
28	7.16	47.05	6.22	7.57	47.32	49.99	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
29	7.44	47.60	6.03	7.64	47.07	48.56	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
30	7.01	48.40	6.16	7.29	46.98	48.61	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
31	7.37	47.86	5.79	6.94	46.37	50.18	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
32	6.90	48.08	6.07	7.84	46.61	49.09	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
33	7.58	47.18	6.34	7.00	46.09	50.40	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
34	7.48	47.13	6.12	7.17	44.35	48.99	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
35	6.80	49.09	6.68	7.47	45.86	48.67	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	
36	8.43	48.35	6.25	6.95	45.37	50.22	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25	

Figure 3.6. Workbook 2 to estimate crude protein (CP) measures of variation of feeds formulated by the 2-bin method

	A	B	C	F	G	H	I	J	K	L	M	N	O	P
1		Corn	SBM	Poultry fat	Limestone	DCP	Vitamin premix	Mineral premix	salt	DL-Met	MIN (Nutrient)	MAX (Nutrient)	Supplied	Average content
2	Cost ($)	16.00	28.00	34.00	3.00	20.00	370.00	57.00	2.78	220.00				
3	weight	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4	CP	6.90	47.51	0.00	0.00	0.00	0.00	0.00	0.00	57.52	23.00	100	23.00	23.00
5	CP SD	0.59	1.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.87	0.87
6	ME	3.35	2.44	8.20	0.00	0.00	0.00	0.00	0.00	3.61	3.20	100	3.20	3.20
7	Ca	0.02	0.27	0.00	38.00	21.30	0.00	0.00	0.30	0.00	1.00	100	1.00	1.00
8	NPP	0.13	0.40	0.00	0.00	18.70	0.00	0.00	0.00	0.00	0.45	100	0.45	0.45
9	TSAA	0.29	1.28	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.90	100	0.90	0.90
10	Met	0.14	0.62	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.50	100	0.56	0.56
11	Cysteine	0.15	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.34	0.34
12	Lysine	0.20	2.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10	100	1.28	1.28
13	Arginine	0.32	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	100	1.55	1.55
14	Valine	0.32	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90	100	1.08	1.08
15	Tryptophan	0.06	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	100	0.29	0.29
16	phenylalanine	0.33	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	100	1.15	1.15
17	Threonine	0.24	1.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	0.87	0.87
18	isoleucine	0.23	2.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	0.99	0.99
19	Histidine	0.19	1.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	100	0.58	0.58
20	sigma^2*Xj^2	0.08	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
21	Quantities	0.48	0.41	0.07	0.02	0.01	0.00	0.00	0.00	0.00				
22	MIN (Ingredien	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
23	MAX(Ingredier	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00				
24	Cost/ Ingredie	7.63	11.53	2.44	0.05	0.24	0.93	0.09	0.01	0.53				
25	Formula cost $	23.45												
26	Z value	0.00												
27	Probability	0.50												

SPM | Stochastic | Outputs

Figure 3.7. Stochastic programming workbook (Workbook 3) based on the 1-bin method

	A	Low Corn	High Corn	Low SBM	High SBM	Poultry fat	Limestone	DCP	/itamin premi	Mineral premi	salt	DL-Met	AIN (Nutrient	AAX (Nutrient	Supplied	verage conten
2	Cost ($)	16.00	16.00	28.00	28.00	34.00	3.00	20.00	370.00	57.00	2.78	220.00				
3	weight	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4	CP	6.40	7.39	46.41	48.66	0.00	0.00	0.00	0.00	0.00	0.00	57.52	23.00	100	23.00	23.24
5	CP SD	0.36	0.37	0.85	0.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.53	0.53
6	Corn CP	-1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
7	Low Corn	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.24	0.00
8	High Corn	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.24	0.00
9	SBM CP	0.00	0.00	-1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
10	High SBM	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.21	0.00
11	Low SBM	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.21	0.00
12	ME	3.35	3.35	2.44	2.44	8.20	0.00	0.00	0.00	0.00	0.00	3.61	3.20	100	3.20	3.20
13	Ca	0.02	0.02	0.27	0.27	0.00	38.00	21.30	0.00	0.00	0.30	0.00	1.00	100	1.00	1.00
14	NPP	0.13	0.13	0.40	0.40	0.00	0.00	18.70	0.00	0.00	0.00	0.00	0.45	100	0.45	0.45
15	TSAA	0.29	0.29	1.28	1.28	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.90	100	0.90	0.90
16	Met	0.14	0.14	0.62	0.62	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.50	100	0.56	0.56
17	Cysteine	0.15	0.15	0.65	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.34	0.34
18	Lysine	0.20	0.20	2.88	2.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10	100	1.30	1.30
19	Arginine	0.32	0.32	3.40	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	100	1.57	1.57
20	Valine	0.32	0.32	2.25	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90	100	1.09	1.09
21	Tryptophan	0.06	0.06	0.64	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	100	0.29	0.29
22	phenylalanine	0.33	0.33	2.40	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	100	1.16	1.16
23	Threonine	0.24	0.24	1.83	1.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	0.88	0.88
24	isoleucine	0.23	0.23	2.14	2.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	1.00	1.00
25	Histidine	0.19	0.19	1.19	1.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	100	0.59	0.59
26	sigma^2*Xj^2	0.01	0.01	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
27	Quantities	0.24	0.24	0.21	0.21	0.07	0.02	0.01	0.00	0.00	0.00	0.00				
28	MIN (Ingredie	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
29	MAX(Ingredie	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00				
30	Cost/ Ingredie	3.76	3.76	5.85	5.85	2.47	0.05	0.24	0.93	0.09	0.01	0.52				
31	Formula cost $	23.52														
32	Z value	-0.84														
33	Probability	0.80														

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Figure 3.8. Stochastic programming workbook (Workbook 4) based on the 2-bin method

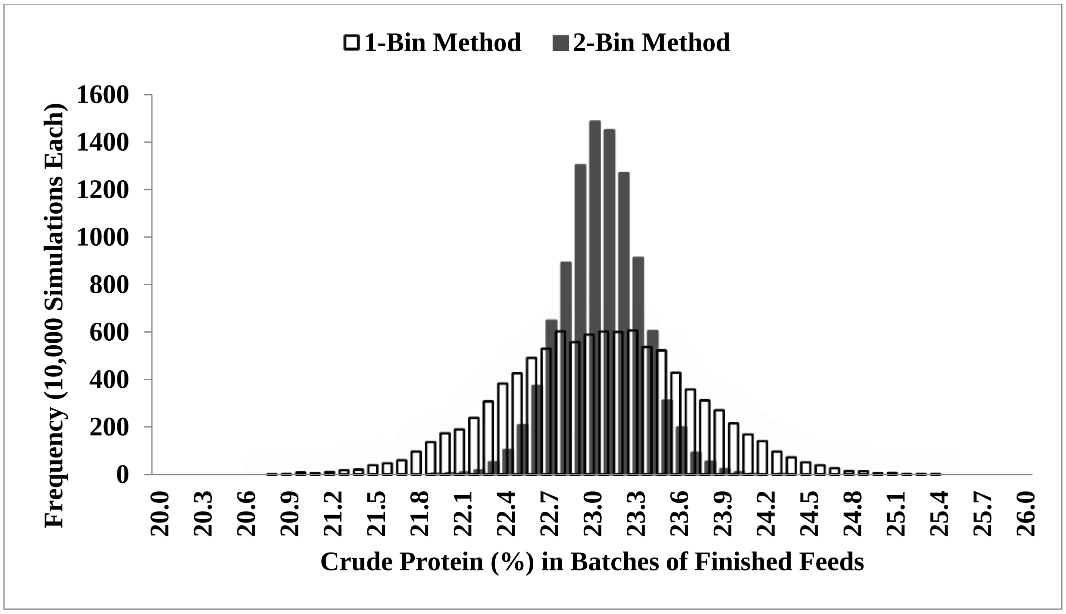

Figure 3.9. Normal distribution curve for crude protein in a broiler-starter feed as affected by grain-handling technique (1-bin versus 2-bin)

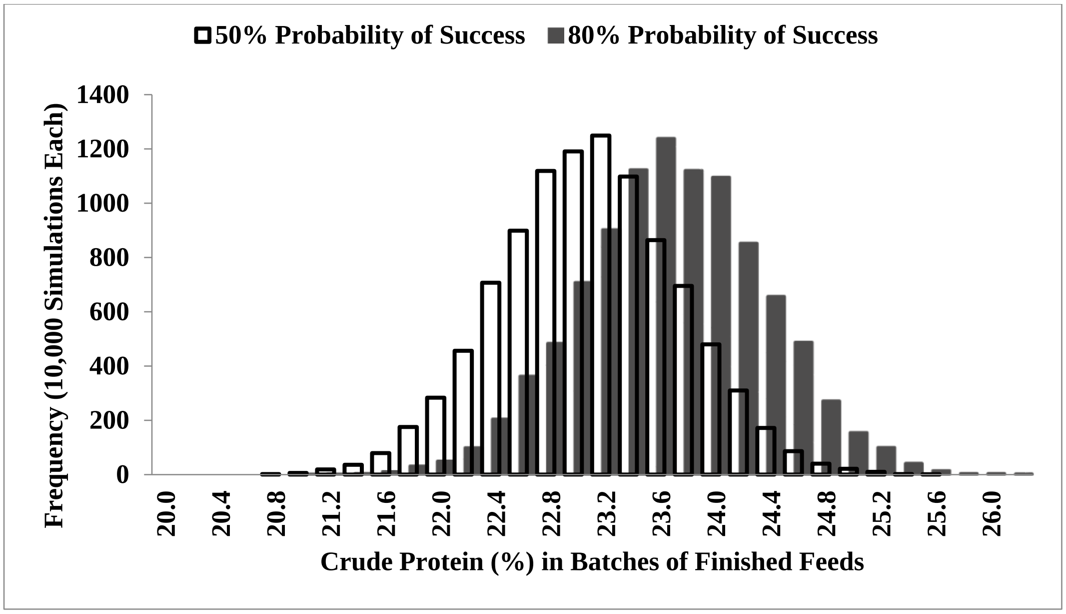

Figure 3.10. Effects of formulating feeds with stochastic programming at 80% probability of success, using the 1-bin method, on the crude protein distribution of a broiler starter feed

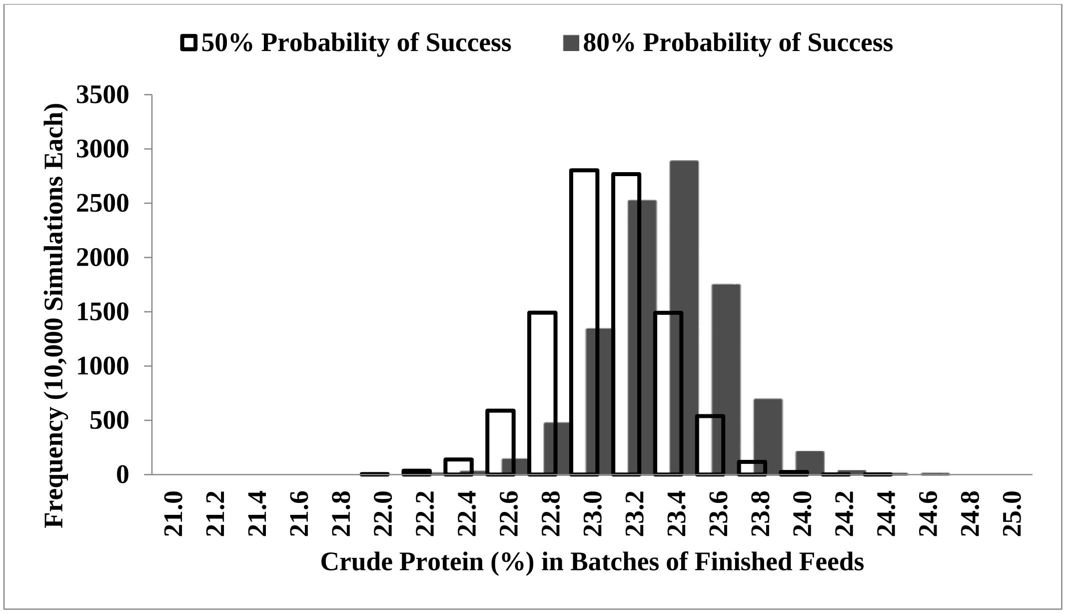

Figure 3.11. Effects of formulating feeds with stochastic programming at 80% probability of success (using the 2-bin method) on the crude protein distribution of a broiler starter feed

CHAPTER 4

QUANTITATIVE ESTIMATES OF THE OPTIMAL BALANCE BETWEEN DIGESTIBLE LYSINE AND THE TRUE PROTEIN CONTENTS OF BROILER FEEDS [1]

[1] Alhotan, R. A. and G. M. Pesti. Accepted by British Poultry Science. Reprinted here with permission of publisher, 04/12/16.

ABSTRACT

1. Typical poultry feed formulation models have been developed for meeting the minimum specifications of the essential amino acids, ignoring the importance of providing precise levels of the non-essential amino acids that are required for maximum performance. Including true protein values in these models in relation to essential amino acids can most accurately account for the requirements of all amino acids in the ration (essential, non-essential and excess essential amino acids).
2. Data from recent research reports on the digestible lysine requirements for maximum weight gain and minimum feed conversion ratio were compiled from the world's literature. Digestible lysine requirements and the true protein contents of the feeds were re-calculated based on common ingredient composition values. Broken-line linear and broken-line quadratic models were fitted to the data and compared.
3. The dLys requirements of broilers (g/kg diet) for body weight gain and feed conversion ratio were found to increase linearly as a function of the true and crude protein contents of the diet. These relationships were not affected by either age or sex. As chickens age the dLys requirements decreased ($P < 0.011$). However, the dLys requirement to true protein ratio did not change with age for body weight gain ($P = 0.135$) or feed conversion ratio ($P = 0.678$).
4. For maximum body weight gain and minimum feed conversion ratio, the dLys requirements were estimated from the prediction models to be 4.92% ± 0.51 and 5.58% ± 0.70 of the true protein level of the diet, using the broken-line linear models, respectively.

5. The good linear relationship between the dLys requirement and true protein level allows the prediction of the variables one from each other for the use in feed formulation to represent the requirements of both essential and non-essential amino acids.
6. The dietary dLys requirements were estimated to be lower using the broken-line linear vs. the broken-quadratic models.
7. True protein was a better predictor of dLys requirements than crude protein (higher R^2 values).

INTRODUCTION

Protein is one of the most important components of feedstuffs considered by nutritionists. Proteins are composed of amino acids which are classified based on dietary needs as essential (EAA) and non-essential amino acids (NEAA). The NEAA can be made in the body from other NEAA or excesses of the EAA (Heger, 2003). The proximate analysis (Kjeldahl or Dumas) Method is commonly used for the determination of the crude protein (CP) content in feedstuffs by multiplying the total nitrogen content of the sample by a conversion factor of 6.25 (assumes 16% nitrogen). The use of CP values obtained by this method in feed formulation is problematic since some nitrogen content comes from other non-protein nitrogen compounds and all proteins do not contain 16% nitrogen. Furthermore, there is a widely held (true) belief that the protein *per se* is not required and the requirements are for its constituent amino acids. This lead to eliminating CP from feed formulation models without a feature to provide for the NEAA needs.

Amino acid requirements vary together since they are required in ratios to make body proteins (Chung and Baker, 1992; Baker and Han, 1994; Mack *et al*., 1999). Amino acids can only be deposited in ratios of amino acids into body proteins (mainly liver and muscle) and the excess cannot be stored and are catabolized (Leeson and Summers, 2001). Ratios are different for maintenance and growth depending on the individual proteins being synthesized. Moreover, amino acid requirements change with age and are subject to molting, antibody production, etc. (NRC, 1994; D'Mello, 2003). Amino acid requirements are also related to growth rate and growth rate is related to gender (male broilers grow faster than females, etc.). Slow vs. fast growing birds show different proportions of growth and maintenance at the same age. While the requirements for the EAA are well-understood, the NEAA requirements are an oxymoron. As the nomenclature implies supplementation of NEAA is not required in the diet but in fact they are absolutely required for protein synthesis as they are essential part of body proteins. So, this raises the question: is there a requirement for total NEAA or amino nitrogen? The answer is yes, or at least amino nitrogen. The historical trend has been to increase synthetic amino acid supplementation while decreasing CP levels of broiler diets to reduce feed costs and nitrogen pollution. The availability of synthetic methionine allowed CP reduction (e.g., more corn less soy in the diet) to meet EAA and NEAA requirements. There has been evidence that when synthetic amino acids are fed in low CP diets there may not be enough NEAA to support the maximum growth and feed conversion (Pesti, 2009). Knowing the importance of the NEAA in poultry nutrition leads to the fundamental question "How to express the NEAA requirements?" The requirements of the NEAA or amino nitrogen have been investigated by evaluating the relationship between the EAA and NEAA (Young and Zamora, 1968; Bedford and Summers, 1985; Wang and Fuller, 1989), EAA and total AA (Ikemoto *et al.*, 1989), EAA nitrogen and NEAA nitrogen (Stucki and Harper, 1961; Lenis *et al*., 1999) and EAA nitrogen and total Nitrogen (Heger *et al.*, 1998; Lenis *et al*., 1999; Evonik, 2005). Other possible ways to represent NEAA "requirements" in feed formulation models include estimating the requirement of excess EAA plus NEAA, the ratio of lysine (or any other amino acid) to CP or the ratio of lysine (or any other amino acid) to true protein (TP; EAA and NEAA components). In practice, the TP content of feedstuffs can be estimated using specific N:P conversion factors known as K_A (the ratio of the weight of the anhydrous AA residues to N content from AA residues; Mosse *et al*.,1985) and K_P (the ratio of the weight of the anhydrous AA residues to total N content; Sosulski and Holt, 1980). The average value of K_A and K_P, called K has been proposed as a good approximation of the relationship between TP and N content (Mosse, 1990). From a scientific point of view, using

CP in feed formulation is problematic as illustrated above, but TP is not. The objectives of this research were 1) to investigate the relationship between digestible lysine (dLys) requirements to CP and TP for data compiled from literature on lysine requirements of broilers; 2) to investigate the effects of sex and age on dLys requirements; and 3) to develop prediction equations for the requirements of dLys for broiler chickens as a function of TP that can be used in feed formulation to represent the requirements of NEAA.

MATERIALS AND METHODS

Raw data set collection

Research pertaining to lysine requirements (total or digestible) for broiler chickens published after the year 2000 was compiled. Variables reported by the original authors including strain, sex, age, tested lysine levels, parameters tested (body weight gain (BWG) and feed conversion ratio (FCR)) and estimated lysine requirements for each parameter were entered into a Microsoft Excel spreadsheet to create a large data set (Tables 4.1 to 4.5). The reported BWG was converted to daily gain (g). The dLys, CP and TP for each experiment were calculated for each diet within each experiment (Tables 4.2 to 4.5). The protein and lysine estimating (PLE) spreadsheet or PLE spreadsheet (Table 4.6) was developed for this purpose. The dLys, CP and TP were calculated for the PLE spreadsheet using a single set of digestibility values (Ajinomoto Heartland LLC; 2014) and the reported diet composition. In case there was no composition reported for a diet other than the basal, the CP and TP differences due to lysine concentrations between the basal and the diet of interest were added to calculate CP and TP for the diets with increasing lysine concentrations. The TP content was calculated for each feed ingredient by multiplying its nitrogen content by the corresponding ingredient-specific N: P conversion factor or K_A (with the exception of the synthetic amino acids whose nitrogen content was multiplied by 6.25). The K_A values were either obtained from the literature (Mosse, 1990; Mariotti *et al*., 2008; Sriperm *et al*., 2011) or estimated from an established linear relationship between CP and TP of the other feed ingredients whose K_A values are known (in case no K_A values were found in literature for the ingredient). The prediction equation used is TP = 0.873*CP + 0.435 (R^2 = 0.99). The dLys values were calculated using the digestibility coefficients from Ajinomoto Heartland LLC (2014) for the experiments conducted on the basis of total lysine. The dLys requirements for BWG and FCR were determined for each experiment using the Nutritional Response Model Workbook (Vedenov and Pesti, 2008) considering the ascending response is linear (broken-line linear model or BLL) and non-linear (broken-line quadratic model or BLQ).

Final data set selection

The final data set was selected based on several criteria. First, they were all published with broiler strain chickens between 2000 and 2014. Experiments were excluded when the models failed to converge on a requirement due to insufficient data points when the broken line was fitted. Figure 4.1 shows typical experiments that were used and excluded due to failure to converge on a parameter estimate: Experiments 1-1 and 2-2 (Table 4.1) converged on a requirement and were included; Experiments 6-1, 10-2 and 11-2 were typical of ones that failed to converge from not having points on the ascending line or plateau. The final dataset included the variables mean age (days), sex (male, female), dLys requirements based on BLL (g/kg diet), dLys requirements based on BLQ (g/kg diet), CP (g/kg diet), and TP (g/kg diet). The mean age is the average of the minimum and maximum ages when dLys was fed (e.g. for the starter period 0-14 the mean age = 7).

Statistical Analysis

The final dataset was subjected to three-way ANOVA using the GLM procedure of SAS software (SAS Institute, 2010) to test the hypotheses of this study. Two statistical models were evaluated for each parameter with dLys being the dependent variable. Model 1 or the complete model included all the effects of age, sex, CP or TP, two-way interaction effect of age and sex, two-way interaction effect of age and CP or TP, two-way interaction effect of sex and CP or TP and the three-way interaction effect of age, sex and CP or TP. Model 2 or the reduced model included all the terms in the complete model with the exception of the three-way interaction effect of age, sex and CP or TP being dropped out of the complete model. A series of simple linear regression equations were evaluated with dLs requirements being the response variable and each of TP, CP and mean age being the predictors. The linear model assumptions of 1) linearity 2) normality 3) Homoscedasticity and 4) Independence were checked to ensure the validity of each model being used. A probability value of less than 0.05 was considered to be significant.

RESULTS

The mean dLys requirements as estimated by BLL vs. BLQ models

The mean dLys requirements estimated by the BLL model for all the experiments of the final data set were lower than the mean dLys estimated by the BLQ model. On average, the BLL values in g/kg of the diet were found to be lower than the BLQ by 0.87 (9.67 vs. 10.54) and 1.09 (9.88 vs. 10.97) for BWG and FCR, respectively. The mean dLys requirements in g/kg of the diet for male and female broilers as estimated by the BLL model were 9.58 ± 0.19 (n=22) and

9.82 ± 0.39 (n=7) for BWG and 9.65 ± 0.22 (n=22) and 10.57 ± 0.43 (n=7) for FCR. The mean dLys requirements in g/kg of the diet for the males and females estimated by the BLQ model were 10.49 ± 0.20 (n=22) and 10.47 ± 0.49 (n=7) for BWG and 10.69 ± 0.24 (n=22) and 11.73 ± 0.56 (n=7) for FCR.

Evaluation of the models fitted to the dLys requirements for BWG and FCR

When evaluating the complete model to fit the dLys requirements for BWG the variables age, sex and TP and their interaction terms were not significantly ($P \geq 0.05$) different than zero (Table 4.7). However, when the three-way interaction term of age by TP by sex was not included as in the reduced model, the variable TP was found be significant (P =0.011). Similarly, all the variables and the interaction terms in the complete model used to fit the dLys requirements for FCR were not different than zero but the variable TP in the reduced model was found to be different (P =0.007) than zero after eliminating the three-way interaction term (Table 4.7). The variable CP in the reduced model for BWG and FCR followed the same trend as with TP and was found to be only significant (for BWG P =0.033; for FCR P =0.029) when the three-way interaction term of age by CP by sex was not included (Table 4.8). The dLys requirement for BWG increased linearly as a function of the TP ($P < 0.001$; R^2=0.77) and CP ($P < 0.001$; R^2=0.72) contents of the diets (Figures 4.2 and 4.3). The dLys requirements for BWG was estimated from the regression equations to be ~ 4.92% ± 0.51 of the TP (dLys = 0.04919*TP; intercept estimate was not different from zero, P = 0.635) and ~ 4.18% ± 0.50 of CP (dLys = 0.04178*CP; intercept estimate was not different from zero, P = 0.311). Similarly, a linear increase ($P < 0.001$) was observed in dLys requirements for FCR as a function of TP (R^2 = 0.70; Figure 4.4) and CP (R^2= 0.58; Figure 4.5). The dLys requirements was estimated to be ~ 5.58% ± 0.70 of TP (dLys = 0.05579*TP; intercept estimate was not different from zero, P = 0.678) and

4.49% ± 0.73 of CP (dLys = 0.04492*CP; intercept estimate was not different from zero, *P* = 0.672). As broilers get older, the dLys requirements for BWG (R^2 = 0.22; Figure 4.6) and FCR (R^2 = 0.22; Figure 4.7) decrease in a linear manner. The dLys requirements as a ratio of TP for BWG (R^2 = 0.08; Figure 4.8) and FCR (R^2 = 0.006; Figure 4.9) did not change with age.

DISCUSSION

Evaluation of the accuracy of the protein and lysine estimating model

The TP content of the feed ingredients (other than the synthetic amino acids) was estimated using the conversion factor K_A. The conversion factor K (average of K_A and K_P) has been regarded as a good approximation of the TP (Mosse, 1990). However, in a recent study (Sriperm *et al*., 2011) the K_A values produced more accurate estimates for TP for selected feed ingredients samples collected from different regions of the USA and analyzed for AA contents in 2009. In their study, the average TP content for corn and SBM as calculated by the summation of the total amino acid residues from the amino acid analysis were 76.7 and 498.2 g/kg of each ingredient, respectively. The average K_A and K values were 5.68 and 5.37 for corn and 5.64 and 5.39 for SBM. To compare the TP content for corn and SBM using either the K_A or the K values reported above, the average corn and SBM of the basal diets in the raw data set in the current study were calculated to be 58 and 17%, respectively. On average, the TP content from corn and SBM combined for the basal diets would have been ~ 119 g/kg if the K_A factor was used and ~ 113 g/kg when using the K factor. Compared to the TP content of 129 g/kg from both ingredients (calculated as TP = 58% * 76.7 + 17% *498.2) based on the sum of the total AA residues from the AA analysis, the TP content from corn and SBM is underestimated by ~ 12% when using the K values and only 8% when using the K_A factor. Corn and SBM are the major suppliers of protein in corn-SBM based diets for broilers and even small deviations in the conversion factors will affect the total TP estimate of the diet. The K_A factor resulted in more accurate estimation of the TP for the ingredients examined. Unlike the K factor, the K_A does not take into account the nitrogen from NPN containing compounds. Therefore, it should remain unaffected by the content of NPN containing compounds in feedstuffs.

The dLys requirements and true protein for BWG and FCR

The coefficient of determination (R^2) values obtained from the fitted relationships of dLys requirements estimated by BLQ as a function of the variables (TP, CP and age) were smaller than those of BLL and did not fit as well. Therefore, the results pertaining to the relationships of dLys estimated by BLQ model and the variables TP, CP, and age were not considered in the scope of the present study since the ratios of the requirements to CP and TP would be more variable and less precise. In general, the dLys requirements estimated by the BLQ model were higher than the BLL model, as expected. The elevated requirements for the BLQ model are attributed to the absence of a clear breakpoint of the requirement in the smooth curve. While there may be theoretical reasons why it is better to consider responses to nutrients non-linear, predicting it is problematical much less precise. The BLL and BLQ models estimated higher dLys requirements for females compared to males. It is well known that the males of the modern broilers grow faster, thus they require more dLys than the females. Our models overestimated the dLys requirements for the females since there are not enough data points (n=7 compared to n=22 for males), particularly at older ages. One of the primary objectives of the present study was to investigate the relationship between dLys requirements and TP of the diet and to apply it in feed formulation if the relationship exists. Protein fed decreased with age, so age and TP are confounded in the data set. Statistically speaking, the two variables are not fixed effects but are related to one another. Thus, the models without the three-way interaction of TP by age by sex demonstrate that the strongest predictors of dLys requirements are TP and CP. There is a good linear correlation between dLys requirements and TP for both BWG ($R^2 = 0.77$) and FCR ($R^2 = 0.70$), providing a better fit to the data compared to CP ($R^2 = 0.72$ for BWG and R^2=0.58 for FCR). We consider the R^2 values obtained from the fitted relationships for TP to be very good taking into account that the data are subject to other sources of variation such as the different experimental conditions, year of study, sex, age, strain etc. The developed simple regression equations allow the prediction of the dLys requirements as a function of the TP (or CP) of the diet and *vice versa*. For maximum BWG and minimum FCR for all broiler strains at all ages, the average dLys requirements were estimated to be 4.92% ± 0.51 and 5.58% ± 0.70 of the TP content of the diets. Similarly, the predicted TP value for maximum BWG was 20.55% ± 2.13 (205.5 ± 21.3 g/kg). Applying the results of the regression equations during feed formulation can fulfill the needs for the NEAA. Theoretically, the needs for the NEAA or amino nitrogen can also be fulfilled by estimating the excess EAA plus NEAA in the diets. The excess of EAA plus NEAA can be expressed in feed formulation models as the nitrogen ratio of the EAA (as a100% of the requirements) to the excess EAA plus NEAA or simply E: N ratio [$N_{\text{EAA (100\% of requirements)}} / N_{\text{Excess EAA (above 100\% of requirements)}} + N_{\text{NEAA}}$]. Heger (2003) suggested using the ratio of EAA nitrogen to total amino acid nitrogen or E: T ratio [$N_{\text{EAA}} / N_{\text{EAA}} + N_{\text{NEAA}}$] as a method to account for the needs of NEAA for optimum performance. He reported a range of E: T ratios (0.55 to 0.60) for optimal growth for studies obtained from literature conducted with different species such as rats, pigs, turkey and chicks. To compare the dLys requirements estimated using the regression equations developed in this study with E: T and E: N methods as described above, corn-SBM diets were formulated to meet or exceed the NRC (NRC, 1994) recommendations of broilers for starter (CP = 23%), grower (CP = 20%), and finisher (CP = 18%) phases. The estimated dLys requirements using the regression equations for minimum FCR were 11.0 g/kg for starter (TP = 20.8%), 9.6 g/kg for grower (TP =18.1%) and 8.6 g/kg for finisher phase (TP = 16.3%). In contrast, the dLys requirements using the method outlined by Heger (2003) were estimated as 11.7 g/kg for starter (E: T = 0.53), 9.7 g/kg for grower (E: T = 0.52) and 8.4 g/kg for finisher phase (E: T = 0.52). The E: N method should produce similar dLys estimates to the E: T method for the three diets but with different ratios. The E: N ratios for starter, grower and finisher phases of the same diets were calculated to be 0.67, 0.71 and 0.69, respectively. It seems more logical that the NEAA needs can most accurately be represented by the E: N ratio rather than the E: T ratio since the nitrogen from excess EAA (as a source of NEAA) should be added to the denominator not the numerator. When comparing the dLys estimates from the regression equation with the two methods, it seems that the dLys estimates based on regression do not deviate substantially.

Relating the dLys requirements to the TP content of the diet should represent both the requirements of EAA and NEAA or amino nitrogen (i.e. excess EAA plus NEAA) if TP is implemented into feed formulation models (e.g. WUFFDA 2.0 in appendix B). Typical feed formulations assure meeting the minimum specification of CP of the ration to represent the sum of EAA, excess EAA and NEAA. However, the use of CP in the feed formulation process is not a precise practice because 1) CP estimates are based on a false assumption that all proteins contain 16% nitrogen and 2) CP estimates may include a significant proportion of the non-protein nitrogen compounds which may not be utilized well by the bird. In contrast, TP can most accurately represent the sum of EAA, excess EAA and NEAA. In this case, the requirements for NEAA or amino nitrogen are met as the excess EAA and NEAA are all accounted for. In order to apply the TP concept in feed formulation: 1) the N content for each feed ingredient to be used in the diet should be estimated, 2) TP can be estimated by multiplying the N content of each ingredient by the corresponding K_A conversion factor and 3) The required level of TP is then specified in the feed formulation model, keeping the dLys requirement set at 4.92% for maximum growth or 5.58% for minimum FCR. Using TP in feed formulations instead of CP should help improve the feed formulation process, leading to maximized profits and minimized N pollution. Future research should focus on determining the biological responses of the birds to excess EAA plus NEAA.

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Table 4.1. Experiments compiled to estimate the relationships between dLys requirements and crude or true protein.

Reference	Data set	Strain	Sex	Age (days)	Comment
Dozier and Payne, 2012	1-1	Ross × Ross 708	Female	1-14	
	1-2	Hubbard × Cobb 500			
Dozier *et al*., 2010	2-1	Ross × Ross TP16	Male	28-42	
	2-2	Cobb × Cobb 700			
Abudabos and Aljumaah, 2010	3-1	Cobb 500	Male	1-10	
	3-2			11-22	
	3-3			22-42	
Mehri *et al*., 2010	4-1	Ross 308	Mixed	15-28	
Dimova *et al*., 2010	5-1	Cobb 500 x Cobb FF	Mixed	35-49	
	5-2	Cobb 500x Hubbard	Male		
Dozier *et al*., 2009a	6-1	Ross × Ross TP16	Male	14-28	
	6-2				
Dozier *et al*., 2009b	7-1	Ross × Ross TP16	Male	14-28	
	7-2		Female		
Berri *et al*., 2008	8-1	Ross 308	Male	21-42	Low Density
	8-2				High Density
Dozier *et al*., 2008	9-2	Ross × Ross 708	Male	49-63	
	9-3		Female		
Plumstead *et al*., 2007	10-1	Ross 344 Male × Ross 308 Female	Male	0-20	22% CP
	10-2				27% CP
Corzo *et al*., 2006	11-1	Hubbard Ultra Yield	Male	42-56	
	11-2		Female		
Garcia *et al*., 2006	12-1 & 12-2	Cobb 500	Male	7-21	Battery & Floor
	12-3 & 12-4		Female		Battery & Floor
	12-5 & 12-6		Male		Battery & Floor
	12-7 & 12-8		Female		Battery & Floor
	12-9 & 12-10		Male		Battery & Floor
	12-11 & 12-12		Female		Battery & Floor
	12-13		Male	21-38	
	12-14		Female		
Sterling *et al*., 2005	13-1	Cobb x Cobb	Male	7-17	17% CP
	13-2				20% CP
	13-3				23% CP
	13-4				26% CP
Greenwood *et al*., 2005	14-1	Cobb-Vantress	Male	16-30	Mash
	14-2				Pellet
Garcia and Batal, 2005	15-1	Cobb 500 byproduct	Male	0-21	
	15-2				
Corzo *et al*., 2003	16-1	Ross × Ross 308	Male	42-56	
Corzo *et al*., 2002	17-1	Ross × Ross 308	Male	42-56	
Labadan *et al*., 2001	18-1	Ross male X Avian female	Male	0-14	
	18-2			15-28	
	18-3			22-42	
	18-4			36-53	
Kidd and Fancher, 2001	19-1	Ross × Ross 508	Male	1-18	
	19-2				

Table 4.2. Ranges of outputs for body weight gain and estimated requirements reported by the authors (data sets in bold are the selected data sets)

Reference	Data set	Original Author			Recalculated Requirements										
					dLys requirements (g/kg)							Max response (g)			
		Req.	Method	Max	BLL[3]			BLQ[4]			Δ[5]	BLL		BLQ	
		(g/kg)[1]	-	(g)[2]	X	± SE	R^2	X	± SE	R^2	X	X	± SE	X	± SE
Dozier and Payne, 2012	**1-1**	12.7	BLQ	382.0	11.8	0.2	98.7	12.9	0.2	99.7	1.1	378.2	2.5	379.6	1.5
	1-2	11.8	BLQ	445.0	10.5	0.2	96.7	11.5	0.3	97.8	1.0	432.4	4.6	435.0	4.1
Dozier *et al*., 2010	**2-1**	9.9	BLQ	1329.0	8.7	0.1	98.1	9.8	0.4	96.9	1.1	1309.8	7.5	1312.0	10.5
	2-2	9.7	BLQ	1479.0	9.5	0.2	96.8	10.5	0.4	97.4	1.0	1452.6	10.2	1454.6	10.0
Abudabos and Aljumaah, 2010	3-1	10.9	NA[6]	287.0	11.5	NA	94.8	11.6	NA	94.9	0.0	282.7	3.7	282.7	3.7
	3-2	9.9	NA	678.0	10.5	NA	98.3	10.6	NA	98.3	0.1	673.3	3.3	673.3	3.3
	3-3	8.4	NA	2010.0	9.1	NA	71.0	9.0	NA	71.0	-0.1	1982.3	NA	1982.3	29.2
Mehri *et al*., 2010	4-1	9.5	BLL	884.0	10.6	1.3	85.7	11.3	2.2	80.7	0.7	852.0	12.9	848.0	17.2
	4-1	10.5	BLQ	884.0											
Dimova *et al*., 2010	5-1	9.6	BLL	3069.0	9.6	0.6	90.2	11.5	1.4	91.7	1.9	3061.0	11.4	3065.5	14.8
	5-2	8.6	BLL	3717.0	9.5	0.2	98.2	10.9	0.5	97.7	1.4	3705.2	4.9	3707.7	6.2
Dozier *et al*., 2009a	6-1	12.3	BLL	956.0	12.4	0.2	99.4	NA	NA	99.2	NA	956.0	17.2	2245.0	NA
	6-2	11.8	BLQ	1062.0	10.1	0.1	98.6	10.9	0.2	99.3	0.9	1055.7	4.4	1056.6	3.2
Dozier *et al*., 2009b	**7-1**	10.9	BLQ	1249.0	10.6	0.1	97.8	11.4	0.3	95.9	0.8	1166.4	5.6	1168.7	8.9
	7-2	9.8	BLQ	969.0	12.3	1.3	58.4	14.3	6.8	57.8	2.0	950.5	12.5	955.0	41.4
Berri *et al*., 2008	8-1	NA	NA	2065.0	12.4	1.3	85.6	13.0	2.2	90.5	0.6	2065.0	11.9	2063.0	12.0
	8-2	NA	NA	1962.0	10.8	NA	88.2	10.8	NA	88.2	0.0	1936.3	18.9	1936.3	18.9
Dozier *et al*., 2008	**9-2**	9.1	QBL	1244.0	7.9	0.2	98.5	9.1	0.6	97.4	1.2	1219.0	11.5	1219.3	17.0
	9-3	NA	NA	NA	6.1	0.2	95.5	6.6	0.4	95.4	0.5	954.4	5.6	954.4	5.6
	9-2	7.9	BLL	1244.0											
Plumstead *et al*., 2007	**10-1**	NA	NA	809.0	9.9	0.1	99.6	10.5	0.3	99.6	0.5	798.0	11.0	798.0	11.0
	10-2	NA	NA	863.0	14.2	0.5	98.6	18.9	7.3	99.1	4.7	863.0	4.1	885.8	50.7
Corzo *et al*., 2006	11-1	8.5	RA[7]	1153.0	8.5	1.5	54.7	9.5	3.2	50.3	1.0	1120.3	22.2	1119.8	32.7
	11-2	NA	RA	883.0	6.2	NA	28.5	6.2	NA	28.5	0.0	851.5	NA	851.5	12.4

[1]dLys requirements reported by the original authors.
[2] Maximum response of BWG reported by the original authors.
[3]Broken-Line (Ascending Linear) Model.
[4]Broken-Line (Ascending Quadratic) Model.

[5]difference between dLys requirements (g/kg) estimated by BLL and BLQ.
[6] Not available.
[7]Regression analysis methodology.

Table 4.3. Ranges of outputs for body weight gain and estimated requirements reported by the authors (continued) (data sets in bold are the selected data sets)

		Original Author			Recalculated Requirements										
					dLys requirements (g/kg)							Max response (g)			
Reference	Data set	Req. (g/kg)[1]	Method	Max (g)[2]	BLL[3]			BLQ[4]			Δ[5]	BLL		BLQ	
			-		X	± SE	R^2	X	± SE	R^2	X	X	± SE	X	± SE
Garcia *et al*., 2006	**12-1**	9.1	OSBL[6]	662.0	10.1	0.4	94.1	11.2	1.3	90.6	1.0	632.0	24.3	634.6	32.4
	12-2	9.3	OSBL	625.0	9.6	0.0	99.9	10.6	0.2	99.8	1.0	623.5	1.5	627.5	6.3
	12-3	9.0	OSBL	656.0	9.1	0.5	73.9	9.4	1.4	73.9	0.3	600.0	29.1	600.0	29.1
	12-4	8.2	OSBL	592.0	9.3	0.1	99.2	9.8	0.3	99.2	0.5	585.0	7.0	585.0	7.0
	12-5	10.1	OSBL	663.0	10.1	0.4	95.1	10.8	0.6	97.2	0.7	658.0	19.3	656.0	17.7
	12-6	9.7	OSBL	587.0	9.6	0.1	99.7	10.3	0.4	98.2	0.7	580.3	4.4	583.2	14.0
	12-7	9.7	OSBL	599.0	10.0	0.5	91.5	10.6	0.8	94.3	0.6	583.0	19.5	579.5	17.3
	12-8	9.7	OSBL	569.0	9.1	0.3	92.8	9.7	0.7	92.9	0.6	540.7	15.2	541.2	16.3
	12-9	NA[7]	OSBL	482.0	8.5	NA	18.1	9.5	4.2	18.8	1.0	457.0	NA	458.3	19.3
	12-10	10.3	OSBL	600.0	9.7	0.2	97.4	10.3	0.5	97.6	0.6	586.0	11.1	586.7	10.1
	12-11	NA	OSBL	469.0	8.4	NA	5.4	9.9	7.7	10.4	1.5	444.0	NA	446.4	21.9
	12-12	9.9	OSBL	547.0	9.0	0.1	98.6	9.4	0.2	98.6	0.4	539.7	4.7	539.7	4.7
	12-13	9.7	OSBL	1660.0	8.8	0.8	85.1	9.4	1.2	89.8	0.6	1625.0	46.0	1618.4	37.5
	12-14	9.3	OSBL	1370.0	8.4	0.7	95.2	9.1	1.2	89.0	0.7	1358.7	6.8	1358.6	12.2
Sterling *et al*., 2005	**13-1**	NA	NA	348.7	7.4	0.0	99.9	8.2	0.5	99.9	0.8	348.7	3.1	352.0	14.5
	13-2	NA	NA	393.0	8.7	0.2	99.5	11.4	4.2	98.3	2.7	393.0	9.6	442.3	130.0
	13-3	NA	NA	441.4	9.6	0.3	90.7	10.4	0.0	98.2	0.9	441.4	14.2	442.0	1.3
	13-4	NA	NA	438.0	10.2	0.1	99.8	11.0	0.6	99.8	0.7	435.2	2.9	436.4	11.9
Greenwood *et al.*, 2005	**14-1**	8.7	ER[8]	835.0	9.4	0.1	98.5	9.7	0.3	98.5	0.4	825.7	4.7	825.7	4.7
	14-2	10.0	ER	870.0	10.4	0.5	90.8	10.2	0.4	97.4	-0.2	865.0	20.3	856.7	8.8
Garcia and Batal, 2005	**15-1**	10.0	BLQ	709.0	10.8	0.2	96.1	11.4	0.5	96.1	0.5	694.0	11.2	694.0	11.2
	15-2	9.9	BLQ	698.0	10.8	0.5	91.8	12.1	1.0	92.7	1.2	672.3	12.9	676.5	14.6
Corzo *et al*., 2003	**16-1**	NA	RA	1227.0	9.0	3.1	40.7	9.5	4.6	31.0	0.5	1187.9	26.1	1185.6	33.27
Corzo *et al*., 2002	17-1	NA	RA[9]	1457	7.9	NA	36.7	7.8	NA	36.7	-0.1	1441.9	NA	1442.0	NA
Labadan *et al*., 2001	18-1	NA	NA	410.0	11.5	0.6	86.6	12.5	1.3	88.4	0.9	402.5	14.3	401.0	14.7
	18-2	NA	NA	801.0	10.9	0.6	90.1	11.8	1.0	93.9	1.0	798.5	12.6	798.3	13.0
	18-3	NA	NA	1483.0	10.8	NA	83.1	12.5	5.8	85.0	1.7	1474.7	NA	1483.9	159.3
	18-4	NA	NA	1457.0	8.7	0.7	82.6	8.3	0.7	89.9	-0.3	1453.5	48.0	1427.7	29.4
Kidd and Fancher 2001	**19-1**	10.7	RA	528.0	10.0	0.0	99.9	10.8	0.3	98.8	0.8	527.0	0.7	528.2	4.97

19-2	11.1	RA	567.0	10.7	0.3	98.0	12.1	0.9	95.7	1.4	557.7	9.7	560.6	17.42

[1]dLys requirements reported by the original authors.

[2] Maximum response of BWG reported by the original authors.

[3]Broken-Line (Ascending Linear) Model.

[4]Broken-Line (Ascending Quadratic) Model.

[5]difference between dLys requirements (g/kg) estimated by BLL and BLQ.

[6] one-slope broken-line methodology.[7]Not available. [8]Exponential regression model. [9]Regression analysis methodology

Table 4.4. Ranges of outputs for feed conversion ratio and estimated requirements reported by the authors (data sets in bold are the selected data sets)

Reference	Dataset	Original Author			Recalculated Requirements										
					dLys requirements (g/kg)							Maximum response (g/g)			
		Req.	Method	Max	BLL[3]			BLQ[4]			Δ[5]	BLL		BLQ	
		(g/kg)[1]	-	(g/g)[2]	X	± SE	R^2	X	± SE	R^2	X	X	± SE	X	± SE
Dozier and Payne, 2012	**1-1**	NA[6]	NA	1.22	12.9	0.2	90.0	14.7	0.80	97.8	1.8	1.22	0.01	1.22	0.01
	1-2	12.6	BLQ	1.16	11.1	0.2	97.0	12.3	0.31	98.6	1.2	1.17	0.01	1.17	0.01
Dozier *et al*. 2010	**2-1**	10.5	BLQ	1.86	9.0	0.30	95.5	10.64	0.65	95.2	1.6	1.90	0.01	1.89	0.02
	2-2	10.1	BLQ	1.77	9.7	0.12	98.9	10.99	0.13	99.8	1.3	1.79	0.01	1.78	0.00
Abudabos and Aljumaah, 2010	3-1	10.9	NA	0.92	11.5	NA	92.6	11.56	NA	92.6	0.1	0.93	NA	0.93	0.01
	3-2	10.8	NA	1.36	10.5	NA	55.3	10.59	NA	55.3	0.0	1.40	0.04	1.40	0.04
	3-3	9.2	NA	1.73	8.9	NA	50.4	9.16	NA	50.4	0.2	1.76	0.03	1.76	NA
Mehri *et al*., 2010	4-1	10.8	BLL	1.55	11.8	1.25	89.1	13.56	4.05	82.2	1.7	1.59	0.02	1.60	0.06
Dimova *et al*., 2010	5-1	9.9	BLL	2.37	10.2	0.25	98.4	11.95	0.70	98.1	1.8	2.39	0.03	2.39	0.04
	5-2	9.1	BLL	2.03	9.6	0.17	98.6	11.20	0.31	99.1	1.6	2.06	0.01	2.05	0.01
Dozier *et al*., 2009a	6-1	12.0	BLL	1.50	12.1	0.17	99.3	14.69	1.00	99.7	2.6	1.50	0.02	1.40	0.08
	6-2	12.4	BLQ	1.49	10.2	0.14	98.8	11.49	0.40	98.4	1.3	1.50	0.01	1.49	0.01
Dozier *et al*., 2009b	**7-1**	11.5	BLQ	1.39	11.0	0.10	98.7	12.05	0.30	98.1	1.1	1.46	0.00	1.45	0.01
	7-2	9.9	BLQ	1.62	10.9	0.42	78.6	10.59	0.42	89.1	-0.3	1.63	0.01	1.64	0.01
Berri et al., 2008	8-1	NA	NA	1.71	10.9	0.18	97.7	11.26	0.51	97.7	0.3	1.71	0.01	1.71	0.01
	8-2	NA	NA	1.75	11.2	0.00	99.9	11.82	0.00	99.9	0.7	NA	0.00	NA	0.00
Dozier *et al*., 2008	**9-2**	8.9	BLQ	2.71	7.8	0.32	95.6	8.92	0.53	97.7	1.1	2.74	0.05	2.74	0.04
	9-3	7.7	BLQ	3.11	7.2	0.59	83.2	7.71	0.92	87.1	0.5	3.21	0.05	3.22	0.04
	9-2	7.8	BLL	2.71											
	9-3	6.2	BLL	3.11											
Plumstead *et al*., 2007	**10-1**	NA	NA	1.59	10.4	0.04	99,9	11.42	0.20	99.9	1.1	1.59	0.01	1.59	0.01
	10-2	NA	NA	1.54	NA	NA		NA	NA		NA	NA	0.00	NA	0.00
Corzo *et al*., 2006	11-1	NA	NA	2.07	8.5	0.95	75.2	9.13	1.62	73.3	1.7	2.11	0.03	2.11	0.04
	11-2	NA	NA	2.26	6.7	0.33	52.2	6.83	1.06	52.2	0.2	2.33	0.03	2.33	0.03

[1]dLys requirements reported by the original authors.
[2] Maximum response of FCR reported by the original authors.
[3]Broken-Line (Ascending Linear) Model.
[4]Broken-Line (Ascending Quadratic) Model.

[5]difference between dLys requirements (g/kg) estimated by BLL and BLQ.
[6] Not available.

Table 4.5. Ranges of outputs for feed conversion ratio and estimated requirements reported by the authors (continued) (data sets in bold are the selected data sets)

Reference	Data set	Original Author			Recalculated Requirements										
					dLys requirements (g/kg)							Maximum response (g/g)			
		Req.	Method	Max	BLL[3]			BLQ[4]			Δ[5]	BLL		BLQ	
		(g/kg)[1]	-	(g/g)[2]	X	± SE	R^2	X	± SE	R^2	X	X	± SE	X	± SE
Garcia *et al*., 2006	**12-1**	9.0	OSBL[6]	1.48	10.0	0.74	94.0	10.49	1.17	89.2	0.5	1.52	0.02	1.53	0.03
	12-2	9.0	OSBL	1.60	10.1	0.29	98.5	10.84	0.05	99.9	0.7	1.60	0.03	1.60	0.00
	12-3	9.3	OSBL	1.37	9.6	0.71	85.0	10.97	1.46	86.5	1.4	1.45	0.04	1.44	0.05
	12-4	9.0	OSBL	1.62	10.1	0.38	97.4	10.69	0.11	99.9	0.6	1.62	0.03	1.62	0.00
	12-5	10.1	OSBL	1.40	10.7	0.53	94.4	11.43	0.64	98.5	0.7	1.40	0.06	1.41	0.04
	12-6	9.9	OSBL	1.44	9.8	0.21	97.6	10.49	0.55	97.1	0.7	1.46	0.02	1.46	0.02
	12-7	10.4	OSBL	1.46	10.4	0.52	93.0	12.15	3.38	89.5	1.7	1.49	0.05	1.46	0.15
	12-8	10.2	OSBL	1.44	9.9	0.33	95.6	10.56	0.49	97.9	0.6	1.45	0.02	1.46	0.02
	12-9	NA	OSBL	1.72	11.3	NA	58.7	9.60	1.33	72.5	-1.7	1.73	NA	1.79	0.05
	12-10	10.8	OSBL	1.40	9.9	0.12	99.4	10.87	0.20	99.7	1.0	1.41	0.01	1.40	0.01
	12-11	NA	OSBL	1.76	9.6	NA	NA	7.98	NA	NA	-1.6	1.82	0.05	1.82	NA
	12-12	11.0	OSBL	1.45	10.0	0.28	97.4	10.78	0.46	98.5	0.8	1.46	0.01	1.46	0.01
	12-13	9.6	OSBL	1.68	8.6	0.38	95.5	9.29	0.44	98.6	0.7	1.69	0.02	1.69	0.01
	12-14	9.6	OSBL	1.78	8.7	0.30	97.4	9.56	0.31	99.4	0.9	1.78	0.01	1.78	0.01
Sterling et al., 2005	**13-1**	NA	NA[8]	1.80	7.0	0.10	99.8	7.74	0.44	99.0	0.7	1.82	0.02	1.81	0.04
	13-2	NA	NA	1.53	8.5	0.25	98.7	9.32	0.03	99.9	0.8	1.53	0.04	1.53	0.00
	13-3	NA	NA	1.40	9.7	0.03	99.9	11.32	0.87	99.6	1.6	1.40	0.00	1.36	0.06
	13-4	NA	NA	1.37	10.8	1.46	75.7	NA	NA	73.6	NA	1.37	0.14	NA	NA
Greenwood *et al*. ,2005	**14-1**	9.0	ER[7]	1.60	9.7	0.07	99.7	10.42	0.18	99.7	0.7	1.60	0.00	1.60	0.00
	14-2	9.9	ER	1.58	9.5	0.10	99.2	10.04	0.23	99.2	0.5	1.60	0.01	1.60	0.01
Garcia and Batal, 2005	**15-1**	11.0	BLQ	1.35	10.7	0.18	95.6	11.03	0.52	95.6	0.3	1.37	0.01	1.37	0.01
	15-2	9.4	BLQ	1.33	11.4	0.34	95.9	12.04	0.31	99.3	0.6	1.34	0.01	1.34	0.00
Corzo *et al*., 2003	**16-1**	NA	RA[9]	2.50	9.3	0.78	80.1	12.92	10.20	81.3	3.6	2.53	0.04	2.46	0.31
Corzo *et al*., 2002	17-1	NA	RA	2.28	7.8	NA	88.9	7.9	NA	88.9	0.1	2.28	NA	2.28	6.12
Labadan *et al*., 2001	18-1	NA	NA	1.29	10.5	0.44	91.1	11.39	0.64	93.5	0.9	1.32	0.03	1.32	0.03
	18-2	NA	NA	1.56	NA	NA	38.6	9.15	1.20	60.1	NA	NA	0.39	1.60	0.03
	18-3	NA	NA	1.76	9.4	0.22	97.3	11.11	1.22	94.6	1.7	1.78	0.01	1.76	0.03
	18-4	NA	NA	2.22	9.7	0.66	92.6	11.83	3.95	94.2	2.2	2.22	0.06	2.17	0.24

Kidd and Fancher,	**19-1**	NA	RA	1.41	9.5	0.09	99.4	10.24	0.20	99.3	0.7	1.42	0.01	1.42	0.01
2001	**19-2**	NA	RA	1.40	10.3	0.10	99.5	11.39	0.49	98.0	1.1	1.42	0.01	1.42	0.02

[1]dLys requirements reported by the original authors.

[2] Maximum response of FCR reported by the original authors.

[3]Broken-Line (Ascending Linear) Model.

[4]Broken-Line (Ascending Quadratic) Model.

[5]diffe+rence between dLys requirements (g/kg) estimated by BLL and BLQ.

[6] one-slope broken-line methodology.

[7]Exponential regression model.

[8]Not available.

[9]Regression analysis methodology.

Table 4.6. Example calculation of the true protein, crude protein and dLys concentrations using the basal diet of Kidd and Fancher (2001).

Ingredient	**CP** [1]	**Lys** [2]	**Ingredient** [3]	**CP Contribution** [4]	**Lys Contribution** [5]	**dLys** [6]	**Total N** [7]	K_A [8]	**TP Contribution** [9]
Corn	84.0	2.6	502.3	42.2	1.3	1.1	13.4	5.68	38.3
Corn gluten meal	624.3	12.5	100.0	62.4	1.3	1.1	99.9	5.50	54.9
Soybean meal	487.2	30.6	196.8	95.9	6.0	5.5	78.0	5.64	86.5
SBM,full fat	376.5	23.8	0.0	0.0	0.0	0.0	60.2	5.53	0.0
Menhaden meal	630.4	47.4	0.0	0.0	0.0	0.0	100.9	5.50	0.0
Peanut meal	448.4	14.5	0.0	0.0	0.0	0.0	71.7	5.52	0.0
Poultry BP meal	577.8	31.5	0.0	0.0	0.0	0.0	92.4	5.45	0.0
Feather meal	843.9	20.3	0.0	0.0	0.0	0.0	135.0	5.49	0.0
Casein	745.8	60.7	0.0	0.0	0.0	0.0	119.3	6.15	0.0
Rapeseed meal	387.5	20.9	0.0	0.0	0.0	0.0	62.0	5.53	0.0
Wheat	150.3	4.1	0.0	0.0	0.0	0.0	24.0	5.49	0.0
Wheat middlings	168.8	6.5	92.7	15.6	0.6	0.5	27.0	5.62	14.1
Sunflower meal	328.2	12.4	0.0	0.0	0.0	0.0	52.5	5.54	0.0
Meat & Bone meal	527.0	27.4	0.0	0.0	0.0	0.0	84.3	5.37	0.0
Triticale	157.7	4.1	0.0	0.0	0.0	0.0	25.2	5.56	0.0
L-Glutamate	590.0	0.0	0.0	0.0	0.0	0.0	94.4	6.25	0.0
L-Arginine	1866.0	0.0	2.2	4.1	0.0	0.0	298.6	6.25	4.1
L-Glycine	1000.0	0.0	0.0	0.0	0.0	0.0	160.0	6.25	0.0
L-Phenylalanine	493.0	0.0	0.0	0.0	0.0	0.0	78.9	6.25	0.0
L-Histidine	650.0	0.0	0.0	0.0	0.0	0.0	104.0	6.25	0.0
L-Valine	747.5	0.0	0.1	0.1	0.0	0.0	119.6	6.25	0.1
L-Isoleucine	687.8	0.0	0.2	0.1	0.0	0.0	110.0	6.25	0.1
L-Lysine HCL	944.0	780.0	0.0	0.0	0.0	0.0	151.0	6.25	0.0
Lysine sulfate	750.0	473.0	0.0	0.0	0.0	0.0	120.0	6.25	0.0
Dl-Methionine	581.0	0.0	3.2	1.9	0.0	0.0	93.0	6.25	1.9
L-Threonine	735.0	0.0	1.2	0.9	0.0	0.0	117.6	6.25	0.9
L-Tryptophan	858.0	0.0	0.0	0.0	0.0	0.0	137.3	6.25	0.0
Calculated Total (%)				223.2	9.2	8.1	2630.4		200.9

[1]Crude protein content (g/kg) for each ingredient based on Ajinomoto Heartland LLC database and the Merck Index (1989).
[2]Total lysine content (g/kg) for each ingredient based on Ajinomoto Heartland LLC database.
[3]Ingredient composition of the diet (g/kg).

[4]Crude protein contribution (g/kg) to the total CP for each ingredient. Calculated by multiplying the CP content of each ingredient by the amount of ingredient used.

[5]Total lysine contribution (g/kg) to the dietary lysine for each ingredient. Calculated by multiplying the total lysine content of each ingredient by the amount of ingredient used.

[6]Digestable lysine for each feed ingredient (g/kg) calculated by multiplying the total lysine content by the corresponding digestibility coefficient from Ajinomoto Heartland LLC database.

[7]Total nitrogen for each feed ingredient (g/kg) calculated by dividing the CP content by 6.25.

[8] Ingredient-specific N : P conversion factor.

[9] True protein contribution (g/kg) from each feed ingredient to the dietary TP calculated by multiplying the total nitrogen of each ingredient by its K_A

Table 4.7. Analysis of variance summary for the two models used to fit the dLys requirements for body weight gain (BWG) and feed conversion ratio (FCR) as a function of age, sex, and true protein.

Source of variation	df	BWG Model		FCR Model	
		Complete[1]	Reduced[2]	Complete[1]	Reduced[2]
		…………… Pr > F[3] ……………			
Age	1	0.126	0.353	0.387	0.275
Sex	1	0.195	0.142	0.499	0.095
True Protein	1	0.231	0.011	0.541	0.007
Age x Sex	1	0.126	0.222	0.389	0.145
Age x True protein	1	0.120	0.604	0.375	0.519
Sex x True protein	1	0.178	0.1269	0.475	0.079
Age x True protein X Sex	1	0.121	-	0.378	-
Error	21(22)[4]				
R^2		0.84	0.82	0.82	0.82

[1] Included all the independent variables, the two-way and three-way interaction terms.
[2] Included all the independent variables and the two-way interaction terms.
[3] Probability values based on Type III sum of squares.
[4] Degrees of freedom in parenthesis are related to the reduced model.

Table 4.8. Analysis of variance summary for the two models used to fit the dLys requirements for body weight gain (BWG) and feed conversion ratio (FCR) as a function of age, sex, and crude protein.

Source of variation	df	BWG Model		FCR Model	
		Complete[1]	Reduced[2]	Complete[1]	Reduced[2]
		……………Pr > F [3]……………			
Age	1	0.190	0.358	0.471	0.285
Sex	1	0.265	0.188	0.571	0.143
Crude Protein	1	0.284	0.033	0.585	0.029
Age x Sex	1	0.191	0.232	0.473	0.173
Age x Crude Protein	1	0.181	0.560	0.457	0.552
Sex x Crude Protein	1	0.241	0.174	0.543	0.126
Age x Crude Protein X Sex	1	0.480	-	0.460	-
Error	21(22)[4]				
R^2		0.78	0.76	0.73	0.73

[1] Included all the independent variables, the two-way and three-way interaction terms.
[2] Included all the independent variables and the two-way interaction terms.
[3] Probability values based on Type III sum of squares.
[4]Degrees of freedom in parenthesis are related to the reduced model.

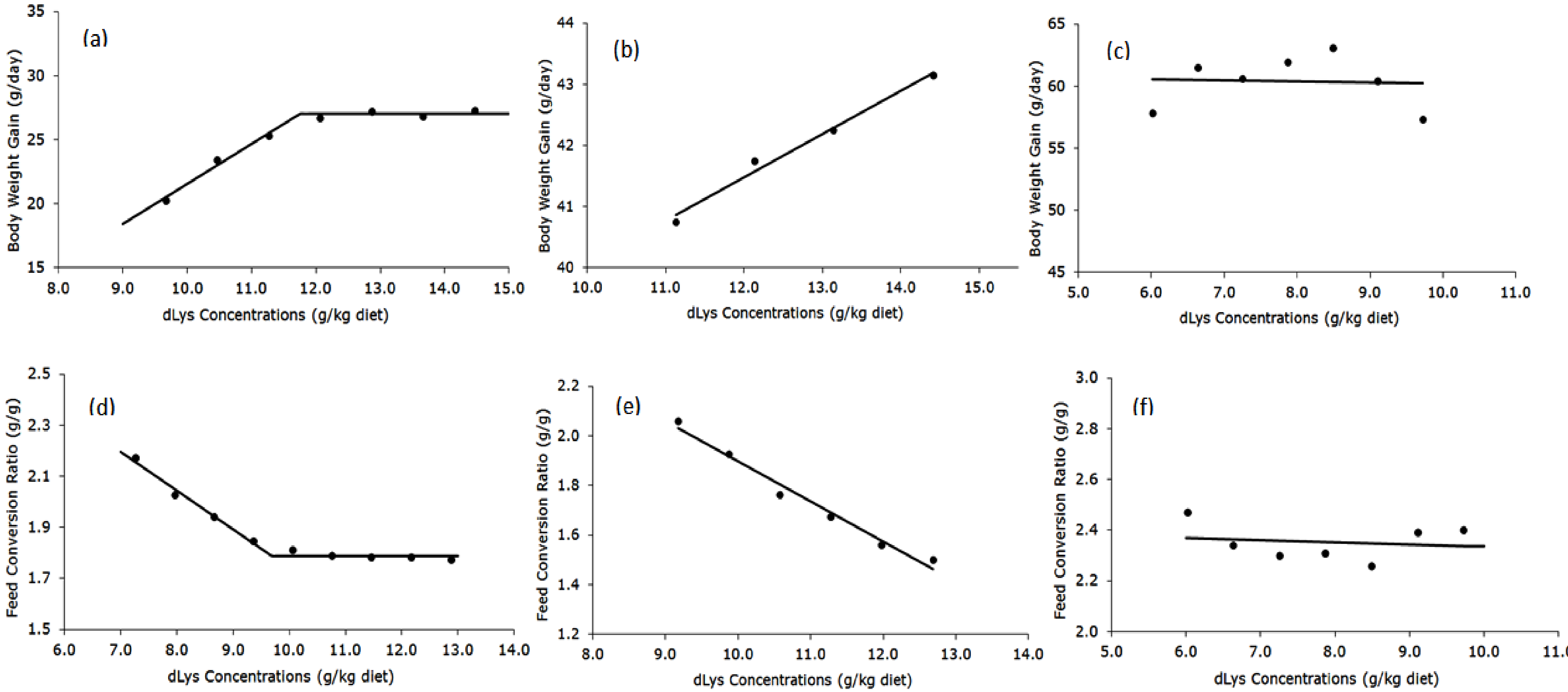

Figure 4.1. Typical experiments that were used and excluded due to failure to converge on a parameter estimate. Graph (a) (data set 1-1) shows an ideal convergence on body weight gain requirement while experiments illustrated in graphs (b) (data set 10-2) and (c) (data set 11-2) failed to converge due to lacking points on the plateau and ascending line, respectively. Graph (d) (data set 2-2) shows an example of convergence on feed conversion ratio requirement. Graphs (e) (data set 6-1) and (f) (data set 11-2) show failure of convergence on feed conversion ratio requirement due to not having points on the plateau and descending line, respectively.

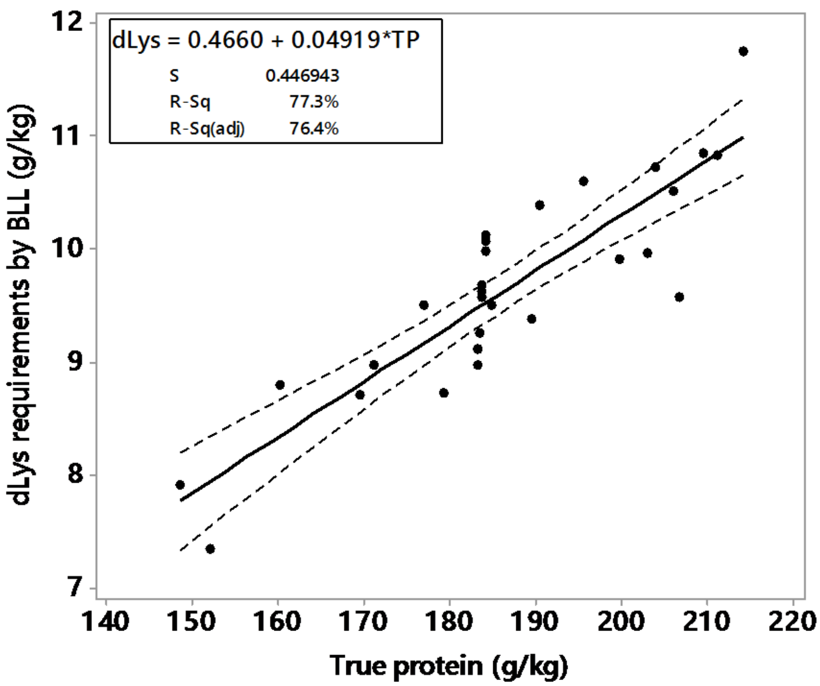

Figure 4.2. Digestible lysine requirements by Broken- Line Linear (BLL) model for body weight gain as a function of the true protein content of the diet (The probability that the slope of the line is equal to zero is <0.001; and the probability that the intercept is equal to zero is 0.635). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

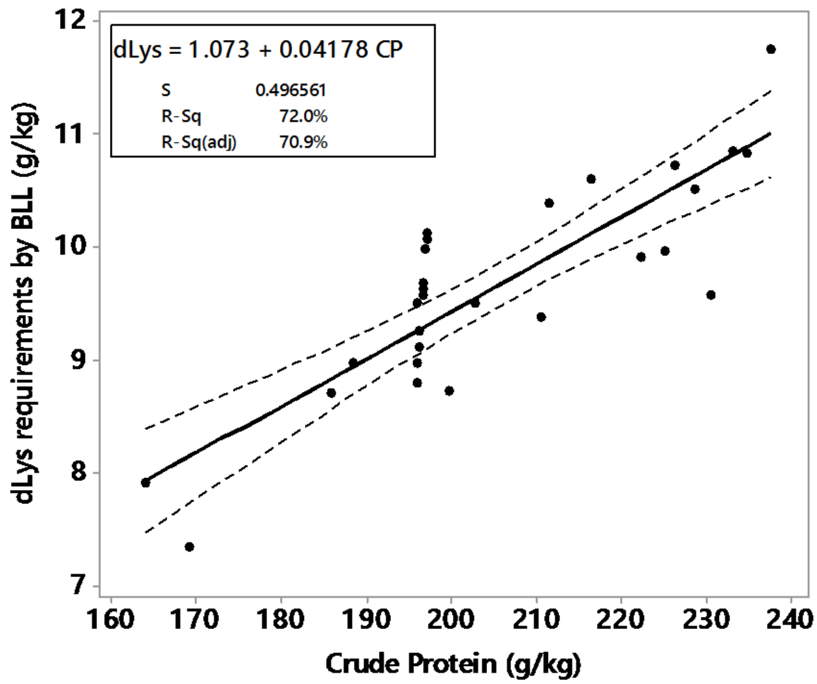

Figure 4.3. Digestible lysine requirements by Broken- Line Linear (BLL) model for body weight gain as a function of the crude protein content of the diet (The probability that the slope of the line is equal to zero is <0.001; and the probability that the intercept is equal to zero is 0.311). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

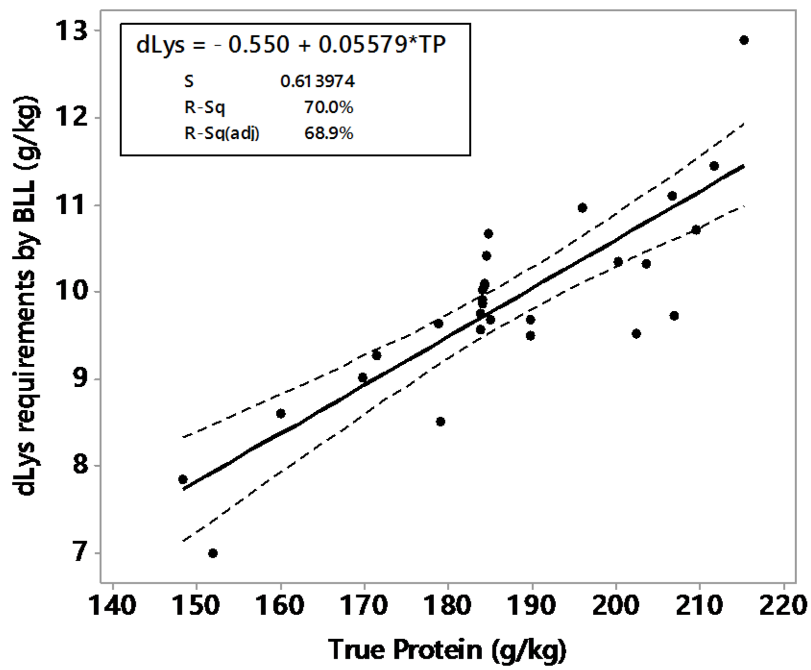

Figure 4.4. Digestible lysine requirements by Broken- Line Linear (BLL) model for feed conversion ratio as a function of the true protein content of the diet (The probability that the slope of the line is equal to zero is < 0.001; and the probability that the intercept is equal to zero is 0.678). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R square.

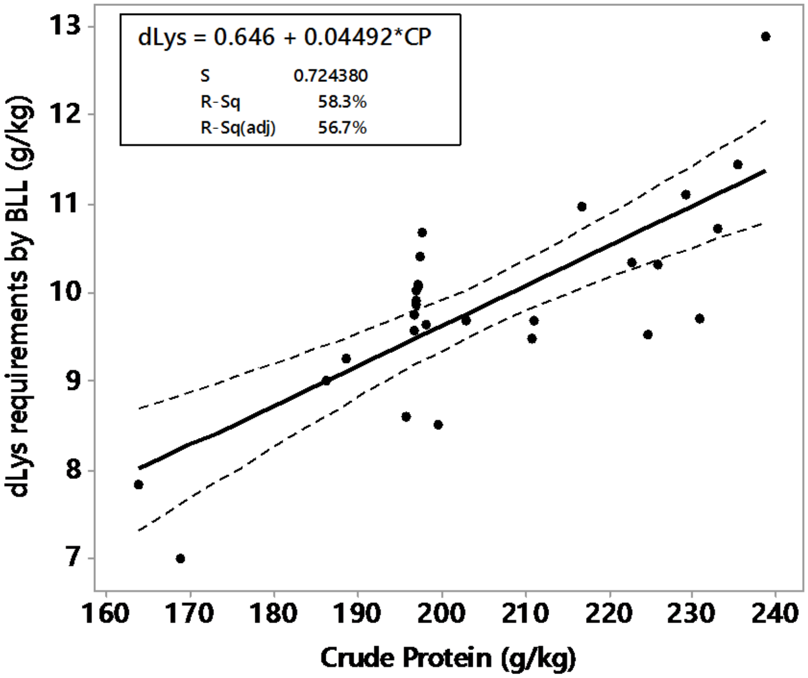

Figure 4.5. Digestible lysine requirements by Broken- Line Linear (BLL) model for feed conversion ratio as a function of the crude protein content of the diet (The probability that the slope of the line is equal to zero is < 0.001; and the probability that the intercept is equal to zero is 0.672). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

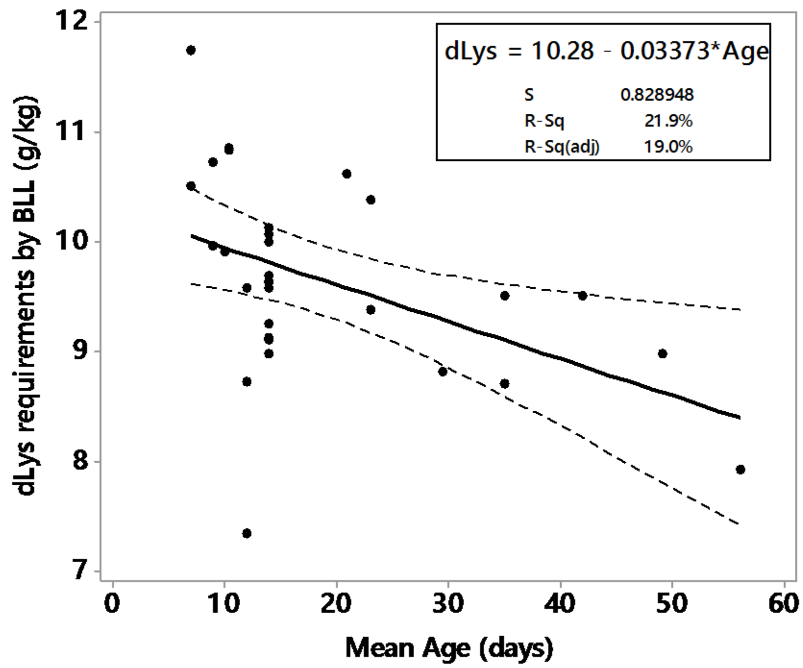

Figure 4.6. Digestible lysine requirements by Broken- Line Linear (BLL) model for body weight gain as a function of age (The probability that the slope of the line is equal to zero is 0.011; and the probability that the intercept is equal to zero is <0.001). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

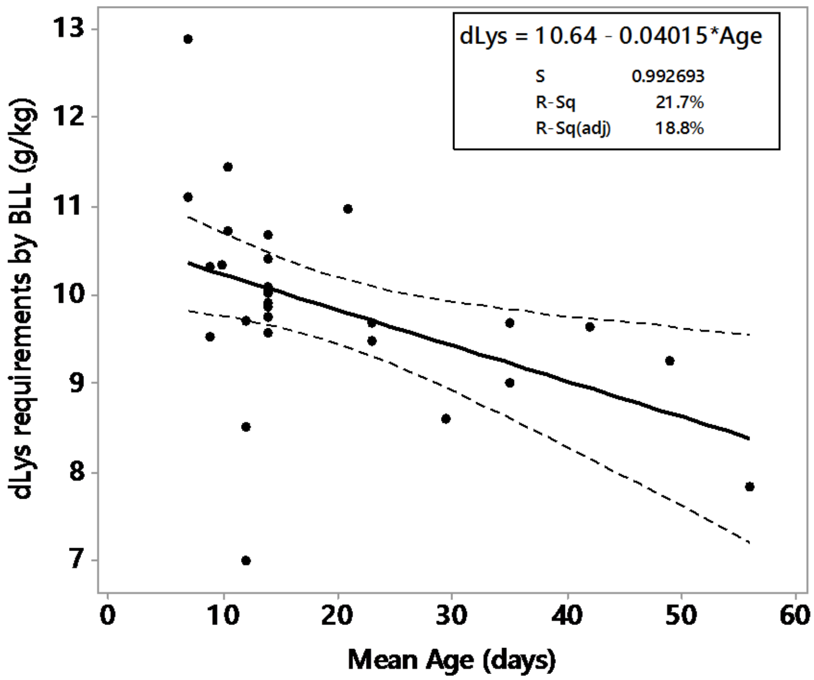

Figure 4.7. Digestible lysine requirements for FCR by Broken- Line Linear (BLL) model for feed conversion ratio as a function of age (The probability that the slope of the line is equal to zero is 0.011; and the probability that the intercept is equal to zero is <0.001). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

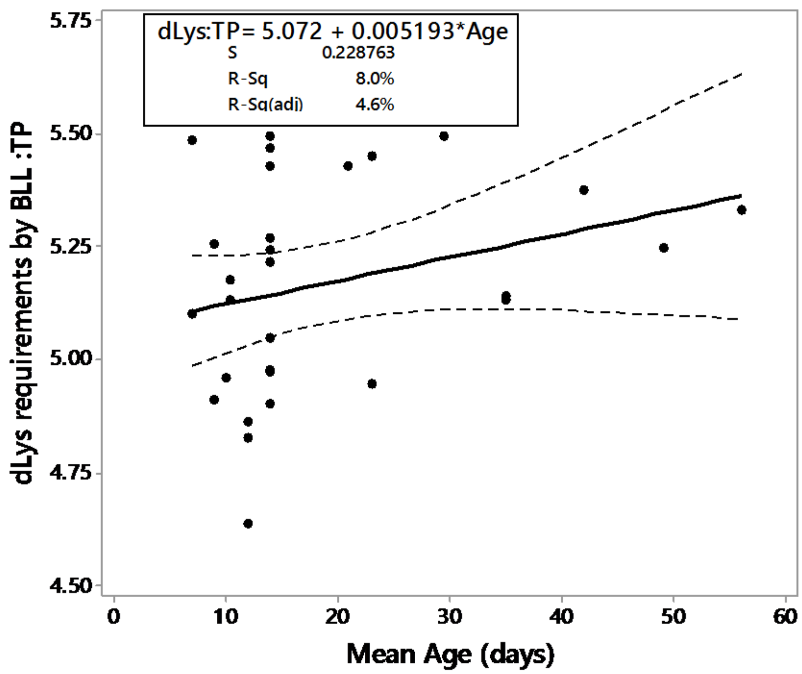

Figure 4.8. The ratio of digestible lysine requirements by Broken- Line Linear (BLL) model to true protein (TP) for body weight gain as a function of age (The probability that the slope of the line is equal to zero is 0.135; and the probability that the intercept is equal to zero is <0.001). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

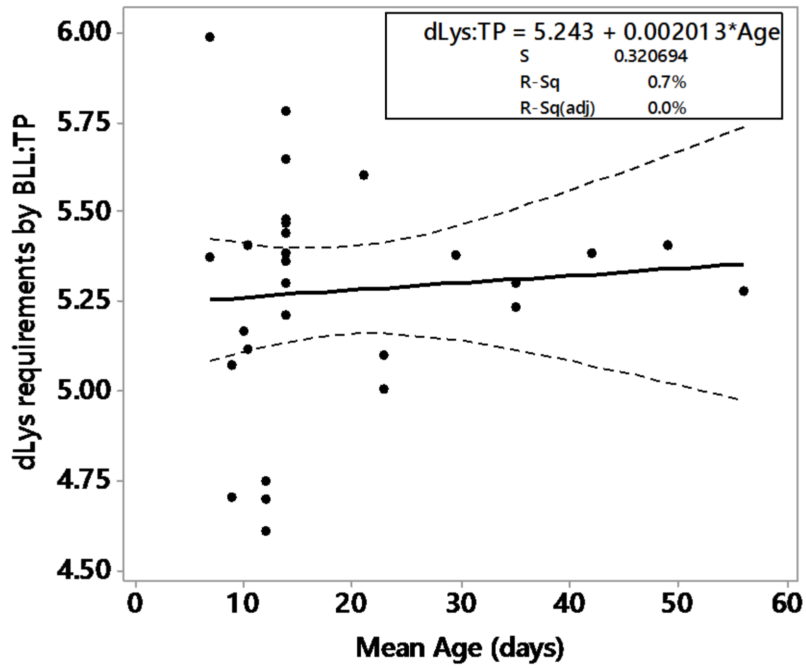

Figure 4.9. The ratio of digestible lysine requirements by Broken- Line Linear (BLL) model to true protein (TP) for feed conversion ratio as a function of age (The probability that the slope of the line is equal to zero is 0.678; and the probability that the intercept is equal to zero is <0.001). S = the square root of the mean square error, R-sq = estimated R-square, R-sq(adj) = estimated adjusted R-square.

CHAPTER 5

TECHNICAL AND ECONOMIC COMPARISONS OF TWO AMINO ACID DATABASES USED IN COMMERCIAL BROILER FEED FORMULATIONS [1]

[1] Alhotan, R. A. and G. M. Pesti. To be submitted to the Journal of Applied Poultry Research.

SUMMARY

A study was conducted to compare the performance, processing yield and returns over feed cost (ROFC) of broiler chickens fed diets formulated using digestible amino acid values from either Ajinomoto Heartland Inc. database (AHI; Chicago, IL; rooster assay) or Evonik Industries database (ED; Hanau-Wolfgang, Germany; chick assay). A total of 720 day-old male broiler chicks were randomly assigned according to dietary treatment to 24 floor pens (12 replicates per diet; 30 chicks per pen). The diets were based on corn, soybean, wheat, DDGS and animal by-product and were formulated to meet the requirements of starting (0-10 d), growing (11-24 d) and finishing (25- 34 d) broiler chickens established by a commercial breeder company. Weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were evaluated at the end of each phase and for the entire period of study. Processing yield (wings, breast and saddle) was evaluated at 35 d. ROFC was calculated for the AHI and ED groups at different scenarios of different feed costs (low, high and moderate) and whole chicken prices ($1.5, $2.2 or $2.9 per kg) and the difference in ROFC was used to compare the groups. There were no significant differences in FI or BWG at any of the experimental periods evaluated. The cumulative FCR (0-34 d) was found to be significantly different with the ED group being more feed efficient. Processing yield measurements did not differ between the groups with the exception of the AHI group having heavier wings and lighter saddles. The ROFC analysis showed that feed formulation using the ED database seems to be more profitable at low to moderate feed costs. When feed costs are low, the value of chicken has to increase to maximize profitability when using the ED database. At high feed costs, the AHI based feed formulation tends to be more profitable at low chicken values and large sizes. The results suggest the

existence of differences in performance and profitability of male broiler production between the AHI and ED databases as they are based on different digestibility assays.

Key words: Broiler, Rooster assay, Chick assay, Amino acid, Feed formulation

DESCRIPTION OF PROBLEM

Modern feed formulation for poultry has been on the basis of digestible amino acids (AAs) after decades of including total AA values in formulation models. The main purpose of this shift was to minimize overfeeding of AA to reduce environmental pollution as well as feed costs. Two assays are often used to determine the digestibility of AAs in feed ingredients, the precision-fed rooster assay and the chick assay. The rooster assay involves force-feeding mature cecectomized roosters the test ingredient in small amounts after a period of fasting then collecting the excreta over the next 48 hrs for AA analysis [1,2]. In contrast, the chick assay involves feeding growing broilers a semi-purified diet *ad libtum* for a period of time (the test ingredient here is the only source of AAs). The birds are then sacrificed and the digesta content of the ilium is collected for the analysis of AAs and the digestible AAs values are standardized by correcting for basal endogenous losses [3]. The use of the digestible AA values from the rooster's assay in broiler feed formulation has been criticized for several reasons such as not feeding a complete diet and fasted roosters are not in a normal physiological state (negative nitrogen balance). In addition, despite the assumption that nutrient digestibility does not change between classes of birds (e.g. leghorn roosters vs. broiler chickens) there is a concern that digestibly values from mature birds may not be applied to growing birds. Garcia et al. [4] determined the digestibility coefficients of AAs in several feed ingredients using the chick and the rooster assays. They reported differences in the digestibility coefficients between the two assays suggesting an age effect or the methodological differences between the assays.

Published reports on digestible AAs for poultry for a variety of feed ingredients are available. There are two commercial amino acid databases known among nutritionists for providing highly accurate data: Ajinomoto Heartland Inc. (AHI; Chicago, IL) and Evonik

Industries (ED; Hanau-Wolfgang, Germany). The AHI database provides digestible AA data based on the rooster assay while the ED database data are based on the standardized chick assay. Although both databases are used widely in broiler feed formulation [5-9], biological and economic comparisons between the databases are scare. Tahir and Pesti [10,11] reported potential savings in feed costs when formulating broiler, turkey and layer diets using digestible AA values from the AHI database over the ED database. Since savings in feed costs may not necessarily reflect net profitability in broiler production, assessing the profitability in terms of technical data is required. Therefore, the objectives of this study were to evaluate the performance and processing yields of male broilers fed diets formulated on the basis of digestible AA values obtained from either AHI or ED database and to conduct an economic comparison of the two databases.

MATERIALS AND METHODS

Experimental Diets

Two experimental diets were formulated to meet or exceed the nutritional requirements for starting (0-10 d), growing (11-24 d) and finishing (25- 34 d) broiler chickens established by a commercial breeding company [12] for broiler chickens (Table 5.1). The two diets were formulated based on the digestible AA values of common feed ingredients obtained from Ajinomoto Heartland Inc. [13] and Evonik Industries [14] databases. The ingredient composition matrix used in the feed formulation was compiled from NRC [15] and the two databases (for CP and digestible AA only) (Table 5.2). The costs of the ingredients used were averages in US market in 2014. Birds were fed the diets *ad libitum* in crumbled form during the starter period and pelleted form during the grower and finisher periods. Samples of the diets were analyzed for total AAs and CP at a commercial laboratory [16,17].

Birds and Housing

The experiment was carried out at the University of Georgia Poultry Research Center (Athens, GA) in an enclosed broiler house. A total of 720 day-old male broiler chicks (Cobb 500 by-products) were obtained from a commercial hatchery [18], weighed and randomly assigned according to dietary treatment to 24 floor pens with 30 chicks each (12 replications per diet). Each floor pen provided a total area of about 3.72 m^2 (0.12 m^2/bird) covered with new wood shavings. In addition, each floor pen was equipped with 1 pan feeder and nipple drinkers. The environment of the house was controlled to assure uniform conditions. The initial temperature of the house was set at 33° C and was reduced gradually to 31° C on day 3 of age and further reduced by 1 degree at days 4, 5, 6, 8, 11, 18, 23, 24, and 26. The lighting program for the first 3 days provided 23L:1D photoperiod with a light intensity of 3.5 foot candles. The lighting program was adjusted after day 3 to provide 20 hours of light with an intensity of 0.5 foot candles. All procedures followed in this experiment were approved by the University of Georgia Institutional Animal Care and Use Committee.

Measurements

Birds and feed were weighed at 0, 10, 24, and 34 day of age to calculate body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). At day 34 of age, a total of 10 birds per pen (120 per treatment; 240 total) were randomly selected, wing banded and moved to the corresponding pen for processing. Hot carcasses were placed in ice water for four hours before deboning. Measurements taken included hot eviscerated carcass weight, chilled carcass weight and the weights of the following chicken parts: wings, breasts (major and minor), saddles (legs and backs) and shells. All parts were weighed and compared to totals for quality control. Eviscerated carcass was expressed as a percentage of live BW while wings, saddles and breast were expressed as percentages of chilled eviscerated carcass.

Economic Analysis

Returns over feed cost (ROFC) was calculated for the AHI and ED groups and the profit difference in ROFC was used to compare the groups. Table 5.3 illustrates the calculation of ROFC from the production functions. Production functions in Figures 5.1 and 5.2 were created from the resulting feed intake and body weight. The production function was used to predict live body weight (kg) from a set of feed intake values ranging between 2.22 to 6.84 kg. Feed cost ($/

bird) was calculated by multiplying the average feed cost $/kg by the total feed intake (kg/Bird). The eviscerated whole chicken weight (kg) was predicted from the live weight using a linear equation relating the eviscerated carcass weights to the live body weights at 35 days of age. The meat value was calculated by multiplying the chilled carcass by the cost of ready-to-cook whole body broiler [19]. The ROFC is the difference between the Meat value and feed cost, and was calculated under different scenarios. The scenarios examined included three formula costs and three chicken prices. The formula costs were: 1) moderate feed costs ($359 per ton for AHI and $370 per ton for ED as the averages of formula costs for the starter, grower and finisher diets in Table 5.1); 2) Low feed costs ($180 per ton for AHI and $185 per ton for ED; calculated as a 50% decrease in the moderate feed costs) and high feed costs ($539 per ton for AHI and $555 per ton for ED; calculated as a 50% increase in the moderate feed costs). The meat prices included $2.2/ kg of meat as an average national price for 2015, a low price ($1.5/kg) and high price ($2.9/kg).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS software [20]. The least significant difference was calculated for each comparison using Fisher's LSD test. P-values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Feed Costs and Diet Composition

Formulating broiler diets based on the ED database in this study resulted in increasing formula costs by $10.95, $9.95 and $9.77/ton for starter, grower and finisher diets, respectively, compared to the AHI database (Table 5.1). The differences in formula costs were mainly due to higher levels of soybean meal and poultry fat used in the ED formulas to meet the minimum protein and energy levels. Pesti and Tahir [10, 11] reported similar cost differences in broiler diets when comparing diets formulated on the basis of AA values originated from ED and AHI databases. The difference in formula cost is not the only difference to be expected between the two databases. Tahir and Pesti reported in another study [21] that the total and digestible AA concentrations in 20 common poultry feed ingredients averaged 6 and 14% higher in the AHI database than in the ED database, respectively. The calculated digestible AAs of the ingredients used in the feed formulation of this study (Figure 5.3) were found to be approximately higher by 8, 7, 22 and 1% for corn, SBM, wheat and DDGS, respectively, for the AHI database than the ED database and this is in agreement with the observation reported by Tahir and Pesti. Intuitively, to meet the minimum specifications of a digestible AA in the case of the ED database, higher concentration of total AA has to be used. As a result, the ED diets should contain more protein than the AHI diets and this is evidenced by the analyzed protein values for the ED diets (Table 5.1) which were higher by 1.95, 2.54 and 0.48% for the starter, grower and finisher diets, respectively.

Live Performance and Processing Yield

The performance results in Table 5.4 did not show any significant differences in feed intake at any of the experimental periods evaluated between the AHI and ED groups. The ED group consumed numerically less feed than the AHI group at all periods. It has been shown that feed intake decreased as the protein level of the diet increased [22-25]. However, the absence of significant differences between the groups in this study could be explained partially by the relatively small differences in protein levels of the diets (1.95, 2.54 and 0.48%) which were not high enough to produce a response. The two groups did not differ ($P > 0.05$) in weight gain at any period (Table 5.4). However, the difference in weight gain at 34 d was approaching significance ($P = 0.08$) with the ED group being heavier by about 26 grams per bird. When comparing the data to the production manual of Cobb 500 male broilers [26] for the same period (0 – 34 d) the ED group was about 18 grams heavier (2,170 vs. 2,152 grams) and the AHI group was about 8 grams lighter (2,144 vs. 2,152 grams) than the standard weights.

The cumulative FCR (0-34 d) was found to be significantly different as the ED groups showing improved FCR during this period (Table 5.4). The ED group had numerically improved FCR during the growing (P=0.051) and finishing (P=0.059) phases. The increase in the final BWG and the decrease in the cumulative feed intake resulted in the improved FCR for the ED group. The improvement in FCR for the ED could be attributed to the high protein levels in the diets. Broiler chickens fed high protein levels were reported to be more feed efficient [22-25]. Both groups had superior feed efficiency compared to Cobb data [26] for the same age (ED: 1.460 vs. 1.539; AHI; 1.488 vs. 1.539).

The mean mortality rate was 2.22 % and was similar between the groups. Processing yield results were similar between the two groups with the exception of the AHI group having heavier wings (as grams per bird and as a percentage of chilled eviscerated carcass) and lighter saddles (%) (Table 5.5).

Economic Analysis

The significant contribution of feed cost in the total production costs makes the cost of feed the major element in determining the profitability for poultry production. For this reason, returns over feed cost (ROFC) is a very useful indicator to assess profitability in poultry production systems. The difference in ROFC between the AHI and ED groups in this study was dependent on feed cost as well as the size and the value of chicken. At moderate feed costs ($359 per ton for AHI and $370 per ton for ED) and a chicken value of $2.2 per kg, the ED group was more profitable than the AHI group and the difference in the ROFC ($ per 1000 chickens) decreased from $22.74 to $20.15 as the size of chicken increased from 1.20 to 2.34 kg and then increased to $20.83 (Table 5.6; Figure 5.4). When chicken meat was valued at $1.5 per kg the profitability for the ED group declined with increasing the size of chicken and become no more profitable at 2.21 kg chicken at which the profits from the AHI group increased as the size increased. At $2.9 per kg of chicken, the difference in ROFC increased greatly as the size increased (especially at medium to large sizes) with the ED group being more profitable. When feed costs were cut by 50% ($180 per ton for AHI and $185 per ton for ED; Table 5.7 and Figure 5.5) the ED group was always profitable than the AHI group at any size and any chicken value. In this scenario, as the size of chicken increased from 1.20 to 2.67 kg the difference in ROFC increased from $19.19 to $25.54 for $1.5/kg, $32.87 to $52.00 for $2.2/kg and $46.55 to $78.46 for $2.9/kg. When feed costs increased by 50% ($539 per ton for AHI and $555 per ton for ED; Table 5.8 and Figure 5.6) the ED group was only profitably for a range of sizes between 1.20 to 2.07 kg when chicken valued at $2.2/kg and all the sizes when the value of chicken increased to $2.9/kg. The AHI group was more profitable at low chicken price ($1.5/kg) and at $2.2/kg but for sizes above 2.07 kg. In general, feed formulation based on the ED database seems to be more profitable at low to moderate feed costs. At moderate feed costs, chicken value has to be increased to maximize profits when using the ED database. When feed costs are high, feed formulation based on AHI database tends to be more profitable at low chicken values and large sizes. It should be noted that these scenarios apply only on male broilers.

The main objectives of the study were to find whether or not there are differences in performance and savings due to using digestible AA values from two databases differing in the assay type (chick assay vs. rooster assay). The difference in FCR between the two group suggests that data from the mature rooster assay may not be applicable to growing chickens. The rooster assay can overestimate the digestibility of some AAs for growing chicks. The economic analysis indicates that the profitability from using the optimal AA database is dependent not only on feed costs but also on chicken value and size. Further research is needed to evaluate the technical and economic differences for female broilers as well as for straight-run flocks.

CONCLUSIONS AND APPLICATIONS

1- The decision to choose which AA database to use in feed formulation will impact the cost of finished broiler feeds due to changes in ingredient usage. The costs of starter, grower and finisher feeds increased by $10.95, $9.95 and $9.77/ton respectively when choosing the ED database (chick assay) over the AHI database (rooster assay) during feed formulation.

2- The decision to choose which AA database to use in feed formulation can impact broiler performance. The ED feeds produced more feed efficient birds than the AHI feeds.

3- Profitability as measured by the difference in returns over feed cost between the AHI and ED groups is dependent on feed cost, chicken size and chicken value. When feed is cheap the ED is more profitable than the AHI and the profitability is maximized as the value and size of chicken increase. When feed is expensive the ED is more profitable only at small sizes and high values.

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Table 5.1. Ingredients and calculated composition of the diets

	Evonik Degussa			Ajinomoto Heartland		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Ingredient, %						
Corn	47.04	51.29	51.43	50.41	54.46	54.74
Soybean Meal -48%	34.78	27.15	20.20	31.94	24.46	17.37
Wheat	5.00	6.00	10.00	5.00	6.00	10.00
DDGS	3.50	4.50	6.50	3.50	4.50	6.50
Animal By Product	3.00	4.00	5.00	3.00	4.00	5.00
DL-Methionine	0.31	0.26	0.21	0.28	0.23	0.19
L-Lysine HCl	0.20	0.18	0.18	0.21	0.20	0.22
L-Threonine	0.08	0.07	0.05	0.07	0.06	0.05
Poultry Fat	3.29	4.40	4.73	2.78	3.91	4.22
Limestone	0.66	0.45	0.40	0.67	0.46	0.40
Defluor. Phos.	1.34	0.91	0.60	1.36	0.93	0.62
Salt	0.40	0.40	0.30	0.40	0.40	0.30
Vitamin Premix [1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix [2]	0.09	0.09	0.09	0.09	0.09	0.09
Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05
Calculated composition [3]						
M.E, kcal/kg	3,030	3,150	3,200	3,030	3,150	3,200
Crude Protein, % [4]	23.55	21.17	19.42	23.55	21.17	19.42
	(24.13)	(22.87)	(20.14)	(22.18)	(20.33)	(19.66)
Calcium, %	1.05	0.90	0.85	1.05	0.90	0.85
Avail. Phos., %	0.50	0.45	0.42	0.50	0.45	0.42
Digestible ARG, %	1.41	1.23	1.08	1.41	1.22	1.06
Digestible HIS, %	0.56	0.50	0.45	0.55	0.49	0.45
Digestible ILE, %	0.87	0.76	0.68	0.87	0.76	0.67
Digestible LEU, %	1.74	1.60	1.50	1.83	1.68	1.56
Digestible LYS, %	1.27	1.10	0.97	1.27	1.10	0.97
Digestible MET, %	0.63	0.55	0.49	0.60	0.52	0.46
Digestible TSAA, %	0.94	0.84	0.76	0.94	0.84	0.76
Digestible PHE, %	1.00	0.88	0.79	1.00	0.88	0.78
Digestible THR, %	0.83	0.73	0.65	0.83	0.73	0.65
Digestible TRP, %	0.24	0.21	0.18	0.24	0.20	0.17
Digestible VAL, %	0.96	0.86	0.79	0.96	0.86	0.78
Feed Cost	385.06	369.44	354.65	374.11	359.49	344.88

[1]Vitamin premix provided the following (per kg of diet): thiamine mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; d-Ca pantothenate, 12 mg; vitamin B12,12.0 µg; pyridoxine-HCl, 2.7 mg; d-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-retinyl acetate, 5,500 IU; all-ractocopherol acetate, 11 IU; ethoxyquin, 150 mg.
[2]Trace mineral premix provides the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5; Se 0.20.
[3] Based on 2007 Broiler nutrient specification by Aviagen (ROSS 308; Aviagen Inc., Huntsville, AL)
[4]Values in parentheses are the analyzed values.

Table 5.2. The feed formulation matrix used to formulate starter, grower and finisher diets based on Ajinomoto Heartland (AHI) and Evonik Industries (ED) databases [1]

	Cost[2] $/ton	Min %	Max %	ME Kcal/g	CP %	Ca %	aP %	dLYS %	dMET %	dTSAA %	dTHR %	dVAL %
Corn AHI	240	0	100	3.35	8.40	0.02	0.10	0.22	0.15	0.32	0.27	0.35
Corn ED	240	0	100	3.35	7.80	0.02	0.10	0.22	0.15	0.30	0.24	0.33
SBM -48% AHI	510	0	100	2.44	48.72	0.27	0.24	2.77	0.61	1.26	1.68	2.05
SBM -48% ED	510	0	100	2.44	46.90	0.27	0.24	2.60	0.59	1.14	1.57	1.94
Wheat AHI	290	-	-	2.80	15.03	0.05	0.11	0.34	0.19	0.50	0.33	0.49
Wheat ED	290	-	-	2.80	11.50	0.05	0.11	0.29	0.16	0.41	0.30	0.44
DDGS AHI	244	-	-	2.48	26.87	0.17	0.41	0.60	0.47	0.86	0.72	1.03
DDGS ED	244	-	-	2.48	26.10	0.17	0.41	0.57	0.43	0.80	0.71	1.02
Animal by product AHI	524	-	-	2.81	55.00	8.50	3.60	2.47	0.75	1.44	1.63	2.32
Animal by product ED	524	-	-	2.81	55.00	8.50	3.60	2.47	0.75	1.44	1.63	2.32
DL-Methionine	3,380	0	100	3.61	58.10	0	0	0	99.00	99.00	0	0
L-Lysine HCl	2,160	0	100	2.81	95.80	0	0	78.00	0	0	0	0
L-Threonine	2,410	0	100	3.15	73.50	0	0	0	0	0	98.50	0
Poultry fat	660	0	100	8.20	0	0	0	0	0	0	0	0
Limestone	48	0	100	0	0	38.00	0	0	0	0	0	0
Defluor. Phos.	494	0	100	0	0	32.00	18.00	0	0	0	0	0
Salt	106	0.40	0.4	0	0	0.30	0	0	0	0	0	0
Vitamin premix	3,600	0.25	0.25	0	0	0	0	0	0	0	0	0
Mineral premix	1,000	0.09	0.09	0	0	0	0	0	0	0	0	0
Coccidiostat	320	0.05	0.05	0	0	0	0	0	0	0	0	0

[1]Values for digestible amino acids and protein are obtained from Ajinomoto Heartland Inc. [13] and Evonik Industries [14] databases. All other values for nutrients were compiled from NRC [15].
[2] Feed costs are averages in US market in 2014.

Table 5.3. An example of Returns over Feed Cost (ROFC) Calculation

Total Feed Intake Kg/Bird	Feed Cost [1] $ / Bird	Live Weight [2] kg	Chilled Carcass [3] Kg	Meat Value [4] $	ROFC [5] $
2.22	1.41	1.63	1.20	3.49	2.08
2.39	1.52	1.73	1.28	3.72	2.20
2.56	1.63	1.83	1.36	3.94	2.31
2.74	1.74	1.93	1.44	4.17	2.43
2.92	1.86	2.04	1.52	4.40	2.54
3.11	1.98	2.14	1.60	4.63	2.65
3.31	2.11	2.25	1.68	4.87	2.76
3.52	2.24	2.35	1.76	5.11	2.87
3.73	2.37	2.46	1.84	5.34	2.96
3.95	2.51	2.56	1.92	5.56	3.05
4.17	2.65	2.66	1.99	5.78	3.14
4.39	2.79	2.75	2.07	6.00	3.21
4.61	2.93	2.85	2.14	6.21	3.27

[1]Feed cost is calculated by multiplying the average feed cost ($/kg) by the total feed intake (kg/Bird).
[2]Live weight (kg) is predicted from a production function relating live weight to feed intake.
[3]Chilled carcass (eviscerated whole chicken) is predicted from the live weight using a linear equation relating the eviscerated carcass weights to the live body weights at 35 days of age.
[4]Meat value is calculated by multiplying the chilled carcass by cost of ready-to-cook whole body broiler.
[5]ROFC is the difference between meat value and feed cost.

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Table 5.4. Effect of feed formulation based on two commercial AA databases on the performance of broiler chickens

	Evonik Industries		Ajinomoto Heartland		Pr > |t|	LSD[1]
Item	Mean	± SEM	Mean	± SEM		
Feed Intake (g)						
0 – 10 d	263.15	3.51	268.49	3.12	0.268	9.74
10 – 24 d	1,301.30	8.11	1,314.60	7.99	0.255	23.61
24 – 34 d	1,605.61	15.15	1,606.95	14.49	0.950	43.47
0 – 34 d	3,170.07	21.88	3,190.05	16.27	0.475	56.54
Body Weight Gain (g)						
0 – 10 d	234.12	3.32	234.35	3.56	0.962	10.10
10 – 24 d	947.99	5.45	941.50	8.96	0.543	21.75
24 – 34 d	987.75	9.38	968.30	10.38	0.176	28.86
0 – 34 d	2,169.86	10.14	2,144.15	9.68	0.080	29.08
Feed Conversion Ratio (g/g)						
0 – 10 d	1.124	0.016	1.147	0.015	0.291	0.045
10 – 24 d	1.373	0.008	1.396	0.008	0.051	0.024
24 – 34 d	1.627	0.010	1.661	0.014	0.059	0.036
0 – 34 d	1.460	0.005	1.488	0.007	0.002	0.017
Mortality Rate (%)						
0 – 34 d	2.22	0.47	2.22	0.47	1.000	1.40

[1] Least significant difference.

Table 5.5. Effect of feed formulation based on two commercial AA databases on processing yield of broilers at 35 days of age

Item	Evonik Industries		Ajinomoto Heartland		Pr > |t|	LSD
	Mean	± SEM	Mean	± SEM		
Live BW (g)	2,188.77	18.01	2,180.89	22.43	0.787	59.65
Eviscerated carcass[3] (g)	1,583.78	14.02	1,563.63	17.60	0.380	46.65
Eviscerated carcass[4] (%)	72.34	0.22	71.71	0.34	0.134	0.85
Wings (g)	170.79	1.98	178.36	2.13	0.016	6.04
Wings[5] (%)	10.48	0.07	10.98	0.09	< 0.001	0.23
Saddles[6] (g)	687.31	7.02	672.04	8.52	0.181	22.90
Saddles[5] (%)	42.20	0.25	41.29	0.19	0.008	0.65
Breast[7] (g)	453.72	7.58	450.02	7.77	0.737	22.52
Breast[5] (%)	27.84	0.33	27.63	0.28	0.632	0.89

[3]Hot eviscerated carcass yield.
[4]Calculated as a percentage of live BW.
[5]Calculated as a percentage of chilled eviscerated carcass.
[6]legs and backs combined.
[7]Pectoralis major and pectoralis minor combined.

Table 5.6. Returns over feed costs (ROFC) from production of broiler chickens fed diets formulated on the basis of Ajinomoto Heartland amino acid database with an average formula cost of $359 per ton or Evonik Industries amino acid database with an average formula cost of $370 per ton (moderate feed costs).

Ajinomoto Heartland				Evonik Industries				Profit Difference per 1000 Chickens[2]		
Whole Chicken, Kg[1]	ROFC for chickens sold at $1.5/ Kg	$2.2/ Kg	$2.90/ Kg	Whole Chicken, Kg[1]	ROFC for chickens sold at $1.5/ Kg	$2.2/ Kg	$2.9/ Kg	$1.5/ Kg	$2.2/ Kg	$2.9/ Kg
1.20	0.86	1.71	2.69	1.22	0.87	1.73	2.73	9.05	22.74	33.96
1.28	0.91	1.81	2.86	1.30	0.92	1.83	2.89	8.39	22.46	33.90
1.36	0.95	1.91	3.02	1.38	0.96	1.93	3.06	7.71	22.20	33.85
1.44	1.00	2.00	3.19	1.46	1.00	2.03	3.22	7.02	21.94	33.83
1.52	1.04	2.10	3.35	1.54	1.04	2.12	3.38	6.32	21.69	33.83
1.60	1.08	2.20	3.51	1.62	1.08	2.22	3.55	5.60	21.45	33.86
1.68	1.11	2.29	3.68	1.70	1.12	2.31	3.71	4.86	21.22	33.92
1.76	1.15	2.38	3.84	1.78	1.15	2.40	3.87	4.11	21.00	34.01
1.84	1.18	2.47	4.00	1.87	1.18	2.49	4.03	3.36	20.81	34.13
1.92	1.20	2.55	4.15	1.94	1.21	2.57	4.18	2.62	20.64	34.30
1.99	1.23	2.62	4.29	2.02	1.23	2.64	4.32	1.89	20.49	34.49
2.07	1.24	2.69	4.42	2.10	1.24	2.71	4.46	1.16	20.37	34.73
2.14	1.26	2.75	4.55	2.17	1.26	2.77	4.58	0.44	20.27	35.01
2.21	1.26	2.81	4.67	2.24	1.26	2.83	4.70	-0.27	20.20	35.33
2.28	1.26	2.86	4.78	2.31	1.26	2.88	4.82	-0.97	20.16	35.69
2.34	1.26	2.90	4.88	2.37	1.26	2.92	4.92	-1.67	20.15	36.10
2.40	1.25	2.93	4.97	2.44	1.25	2.95	5.01	-2.35	20.17	36.56
2.46	1.24	2.96	5.06	2.50	1.23	2.98	5.09	-3.02	20.23	37.08
2.52	1.21	2.98	5.13	2.55	1.21	3.00	5.17	-3.69	20.32	37.65
2.57	1.18	2.98	5.19	2.61	1.18	3.00	5.23	-4.34	20.45	38.27
2.62	1.15	2.98	5.24	2.66	1.14	3.00	5.28	-4.99	20.62	38.97
2.67	1.10	2.97	5.28	2.70	1.10	2.99	5.31	-5.63	20.83	39.74

[1] Whole eviscerated chicken in kg

[2] Calculated as ROFC for Evonik Industries minus ROFC for Ajinomoto Heartland times 1000

Table 5.7. Returns over feed costs (ROFC) from production of broiler chickens fed diets formulated on the basis of Ajinomoto Heartland amino acid database with an average formula cost of $180 per ton or Evonik Industries amino acid database with an average formula cost of $185 per ton (low feed costs).

Ajinomoto Heartland				Evonik Industries				Profit Difference per 1000 Chickens[2]		
Whole Chicken, Kg[1]	ROFC for chickens sold at $1.5/ Kg	$2.2/ Kg	$2.9/ Kg	Whole Chicken, Kg[1]	ROFC for chickens sold at $1.5/ Kg	$2.2/ Kg	$2.9/ Kg	$1.5/ Kg	$2.2/ Kg	$2.9/ Kg
1.20	1.34	2.18	3.02	1.22	1.35	2.21	3.07	19.19	32.87	46.55
1.28	1.42	2.31	3.21	1.30	1.44	2.35	3.26	19.27	33.35	47.42
1.36	1.50	2.45	3.40	1.38	1.52	2.48	3.45	19.38	33.86	48.34
1.44	1.58	2.58	3.59	1.46	1.60	2.62	3.64	19.49	34.41	49.32
1.52	1.66	2.72	3.78	1.54	1.68	2.75	3.83	19.63	35.00	50.37
1.60	1.74	2.85	3.97	1.62	1.76	2.89	4.02	19.78	35.63	51.48
1.68	1.82	2.99	4.17	1.70	1.84	3.03	4.22	19.96	36.31	52.67
1.76	1.89	3.13	4.36	1.78	1.91	3.16	4.41	20.16	37.05	53.95
1.84	1.97	3.26	4.55	1.87	1.99	3.30	4.60	20.38	37.82	55.27
1.92	2.04	3.38	4.73	1.94	2.06	3.42	4.78	20.62	38.63	56.65
1.99	2.11	3.51	4.90	2.02	2.13	3.55	4.96	20.88	39.48	58.08
2.07	2.17	3.62	5.07	2.10	2.19	3.66	5.13	21.16	40.37	59.57
2.14	2.23	3.73	5.23	2.17	2.25	3.77	5.29	21.47	41.29	61.12
2.21	2.29	3.84	5.38	2.24	2.31	3.88	5.45	21.80	42.27	62.74
2.28	2.34	3.93	5.53	2.31	2.36	3.98	5.59	22.16	43.29	64.43
2.34	2.39	4.03	5.66	2.37	2.41	4.07	5.73	22.54	44.36	66.18
2.40	2.43	4.11	5.79	2.44	2.45	4.16	5.86	22.96	45.48	68.01
2.46	2.46	4.19	5.91	2.50	2.49	4.23	5.98	23.40	46.65	69.91
2.52	2.50	4.26	6.02	2.55	2.52	4.31	6.09	23.88	47.88	71.89
2.57	2.52	4.32	6.12	2.61	2.54	4.37	6.19	24.39	49.18	73.96
2.62	2.54	4.37	6.21	2.66	2.56	4.42	6.28	24.94	50.55	76.17
2.67	2.55	4.42	6.28	2.70	2.58	4.47	6.36	25.54	52.00	78.46

[1] Whole eviscerated chicken in kg

[2] Calculated as ROFC for Evonik Industries minus ROFC for Ajinomoto Heartland times 1000

Table 5.8. Returns over feed costs (ROFC) from production of broiler chickens fed diets formulated on the basis of Ajinomoto Heartland amino acid database with an average formula cost of $539 per ton or Evonik Industries amino acid database with an average formula cost of $555 per ton (high feed costs).

Ajinomoto Heartland				Evonik Industries				Profit Difference per 1000 Chickens[2]		
Whole	ROFC for chickens sold at			Whole	ROFC for chickens sold at					
Chicken, Kg[1]	$1.5/ Kg	$2.2/ Kg	$2.9/ Kg	Chicken, Kg[1]	$1.5/ Kg	$2.2/ Kg	$2.9/ Kg	$1.5/ Kg	$2.2/ Kg	$2.9/ Kg
1.20	0.39	1.24	2.08	1.22	0.39	1.25	2.11	-1.08	12.60	26.29
1.28	0.40	1.30	2.20	1.30	0.40	1.31	2.22	-2.49	11.58	25.65
1.36	0.41	1.36	2.31	1.38	0.41	1.37	2.34	-3.95	10.53	25.02
1.44	0.42	1.42	2.43	1.46	0.41	1.43	2.45	-5.45	9.47	24.39
1.52	0.42	1.48	2.54	1.54	0.41	1.49	2.57	-6.99	8.38	23.75
1.60	0.42	1.54	2.65	1.62	0.41	1.54	2.68	-8.59	7.26	23.11
1.68	0.41	1.59	2.76	1.70	0.40	1.59	2.78	-10.23	6.12	22.48
1.76	0.40	1.63	2.87	1.78	0.39	1.64	2.89	-11.94	4.96	21.85
1.84	0.39	1.68	2.96	1.87	0.37	1.68	2.99	-13.65	3.79	21.24
1.92	0.37	1.71	3.05	1.94	0.35	1.71	3.08	-15.37	2.64	20.66
1.99	0.34	1.74	3.14	2.02	0.33	1.74	3.16	-17.10	1.50	20.11
2.07	0.31	1.76	3.21	2.10	0.29	1.76	3.23	-18.83	0.37	19.58
2.14	0.28	1.78	3.27	2.17	0.26	1.78	3.29	-20.58	-0.75	19.08
2.21	0.24	1.78	3.33	2.24	0.21	1.78	3.35	-22.33	-1.86	18.61
2.28	0.19	1.78	3.38	2.31	0.17	1.78	3.40	-24.10	-2.97	18.17
2.34	0.14	1.77	3.41	2.37	0.11	1.77	3.43	-25.87	-4.06	17.76
2.40	0.07	1.76	3.44	2.44	0.05	1.75	3.46	-27.66	-5.13	17.39
2.46	0.01	1.73	3.45	2.50	-0.02	1.72	3.47	-29.45	-6.20	17.06
2.52	-0.07	1.69	3.46	2.55	-0.10	1.69	3.47	-31.25	-7.25	16.76
2.57	-0.15	1.65	3.45	2.61	-0.18	1.64	3.46	-33.07	-8.28	16.51
2.62	-0.24	1.59	3.42	2.66	-0.28	1.58	3.44	-34.93	-9.32	16.29
2.67	-0.35	1.52	3.39	2.70	-0.38	1.51	3.40	-36.79	-10.33	16.13

[1] Whole eviscerated chicken in kg

[2] Calculated as ROFC for Evonik Industries minus ROFC for Ajinomoto Heartland times 1000

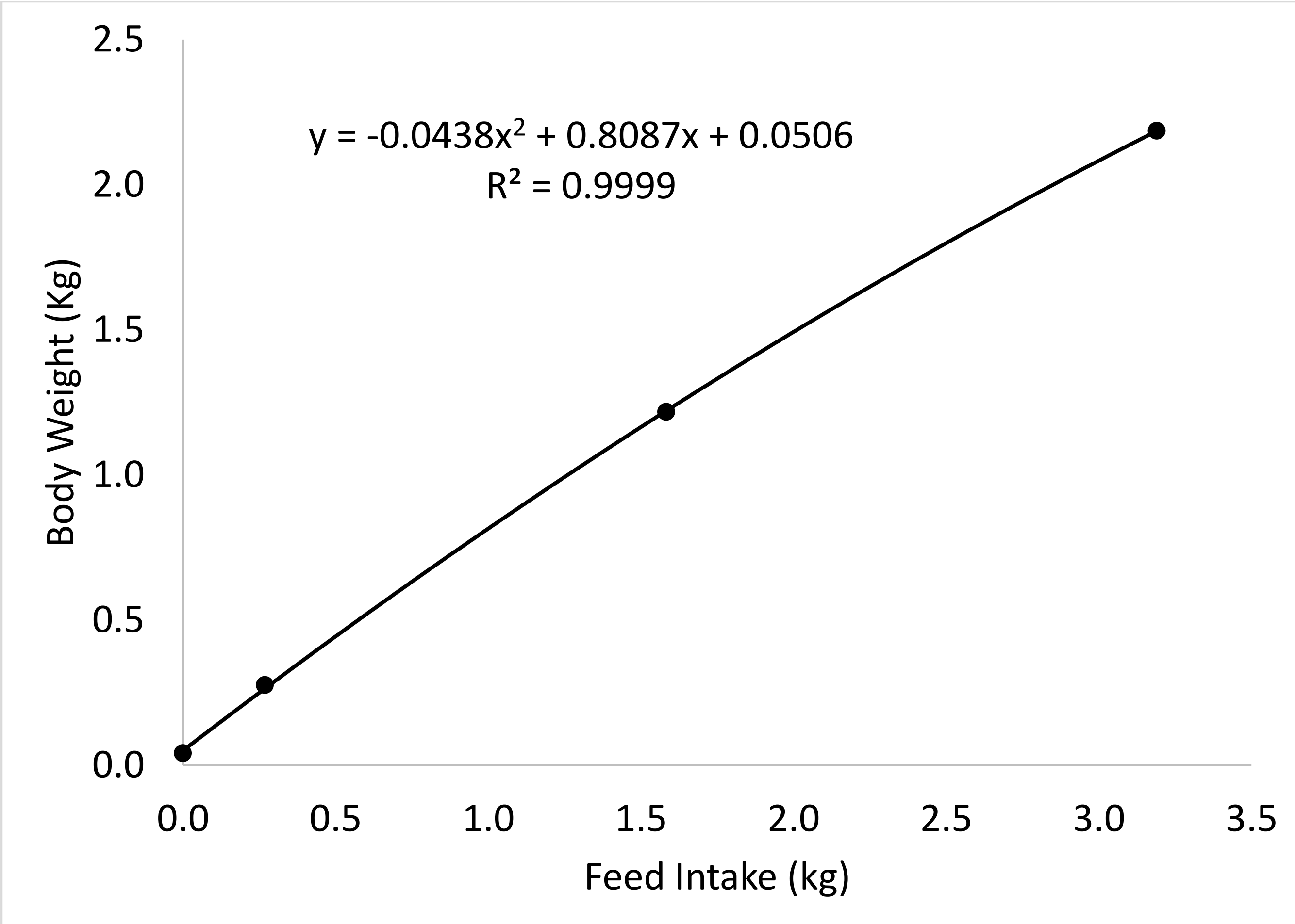

Figure 5.1. Production function for broiler chickens fed diets formulated on the basis of Ajinomoto Heartland database.

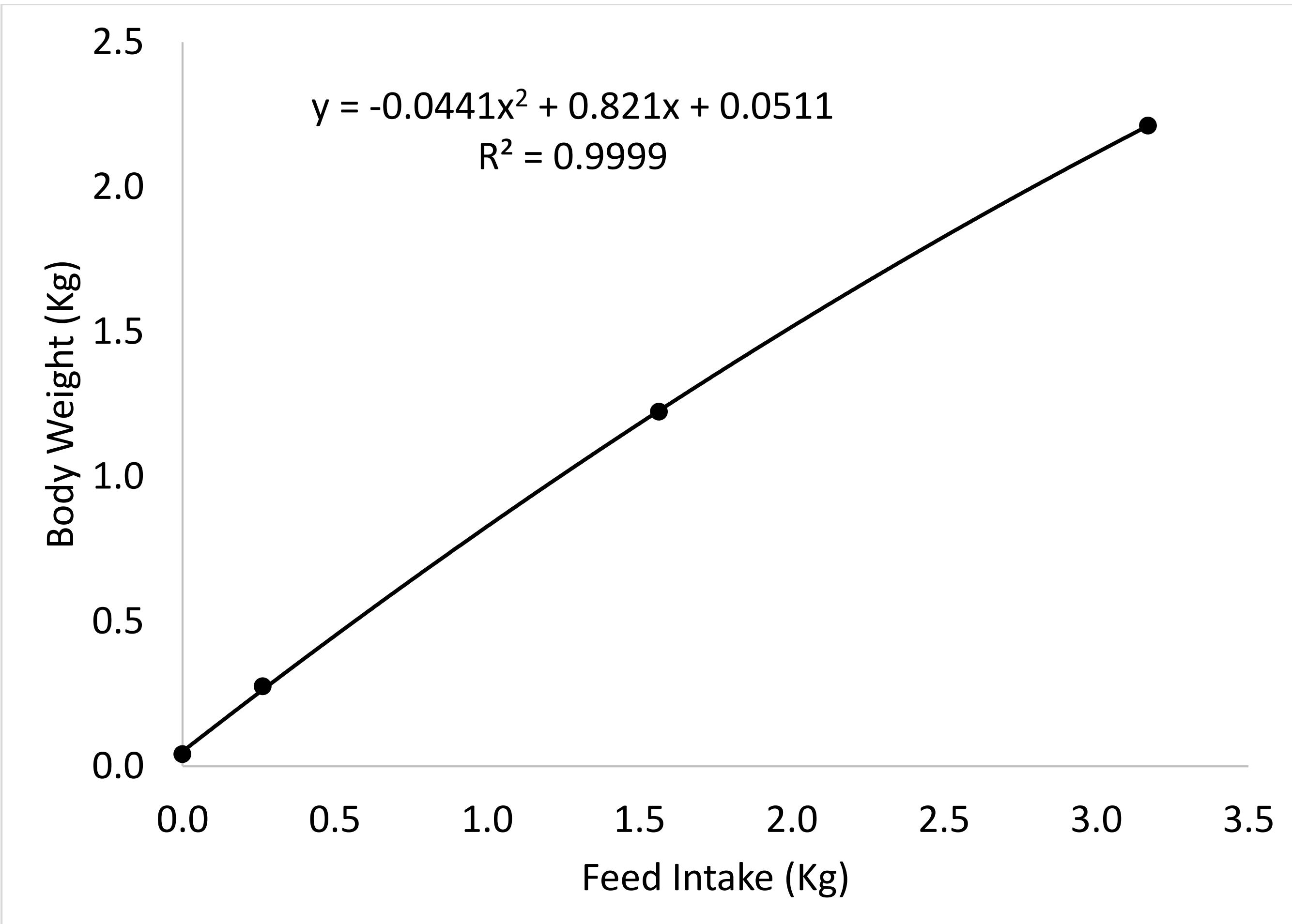

Figure 5.2. Production function for broiler chickens fed diets formulated on the basis of Evonik Industries database.

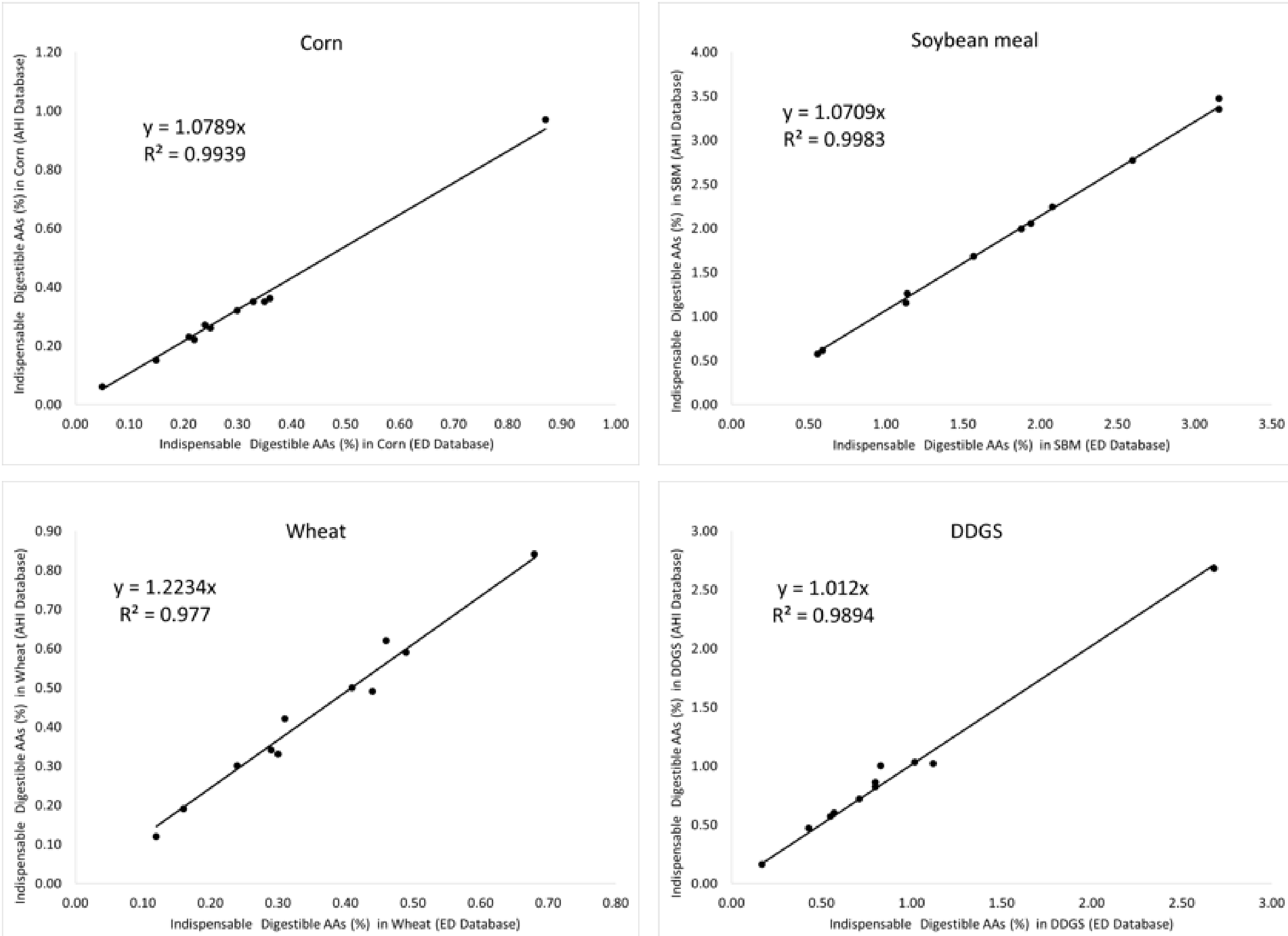

Figure 5.3. The relationship between the indispensable digestible amino acids (% of diet) for the main ingredients used as reported by Evonik Industries (ED) and Ajinomoto Heartland, Inc. (AHI) databases.

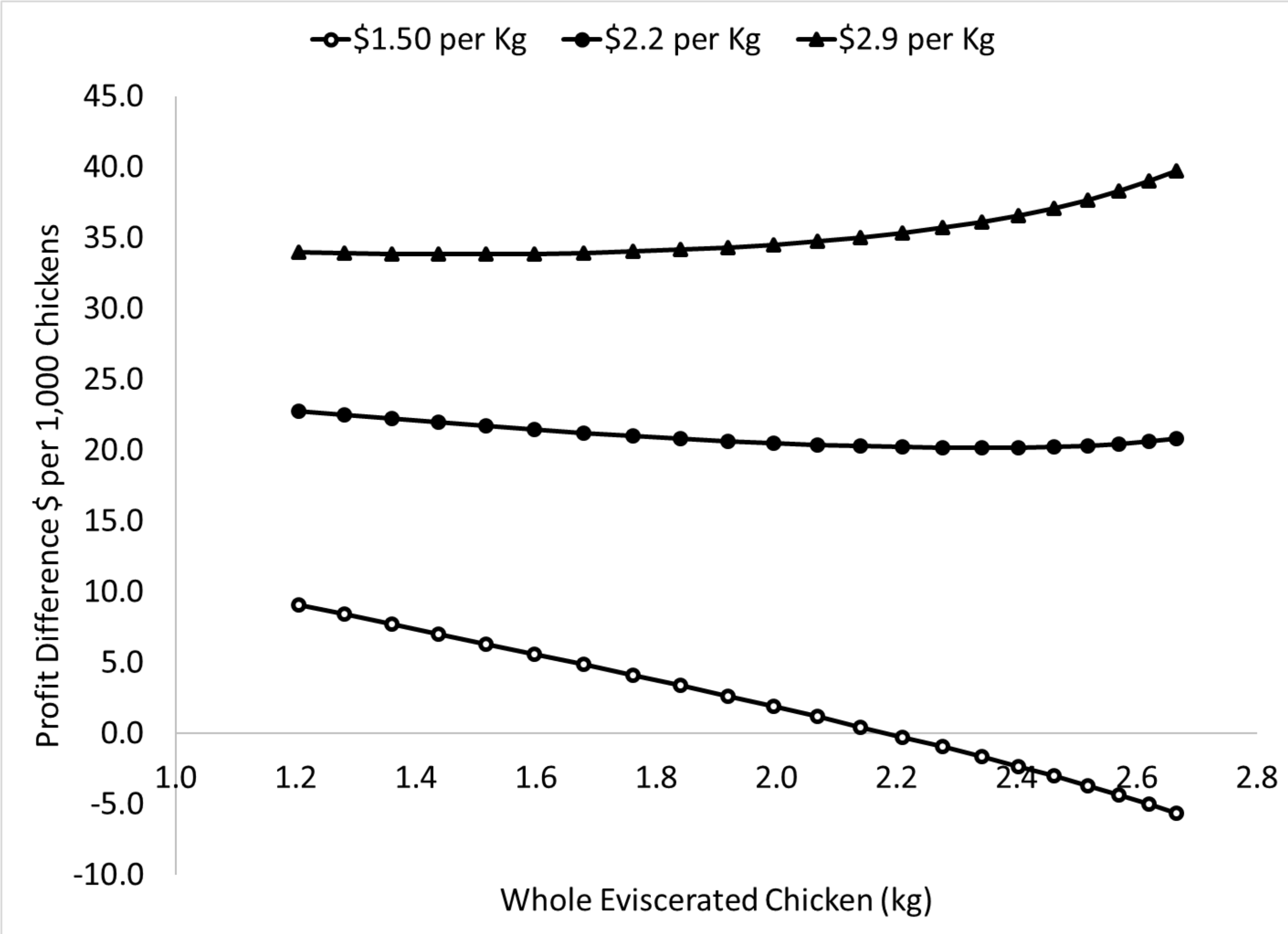

Figure 5.4. Difference in Returns Over Feed Cost (ROFC) at different sizes of chickens sold at three prices ($1.5, $2.2 or $2.9 per kg) from production of male broiler chickens fed diets formulated on the basis of Ajinomoto Heartland (AHI) database with an average formula cost of $359 per ton or Evonik Industries (ED) database with an average formula cost of $370 per ton (moderate feed costs). Profit difference was calculated as ROFC for ED minus ROFC for AHI times 1000. Positive difference indicates potential savings from using the ED database while negative values indicate savings from using the AHI database. All costs and prices are in US dollar.

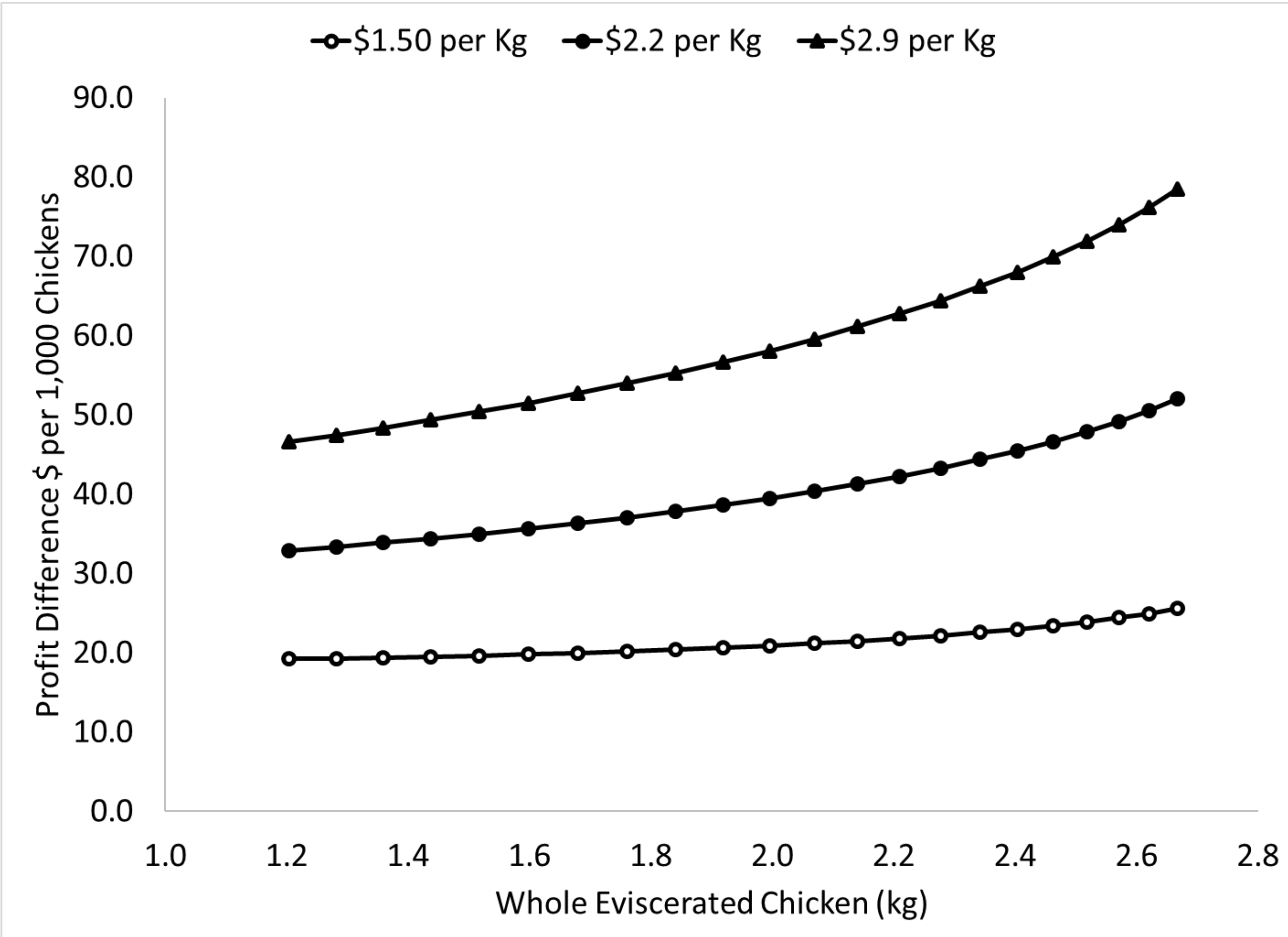

Figure 5.5. Difference in Returns Over Feed Cost (ROFC) at different sizes of chickens sold at three prices ($1.5, $2.2 or $2.9 per kg) from production of male broiler chickens fed diets formulated on the basis of Ajinomoto Heartland (AHI) database with an average formula cost of $180 per ton or Evonik Industries (ED) database with an average formula cost of $185 per ton (low feed costs). Profit difference was calculated as ROFC for ED minus ROFC for AHI times 1000. Positive difference indicates potential savings from using the ED database while negative values indicate savings from using the AHI database. All costs and prices are in US dollar.

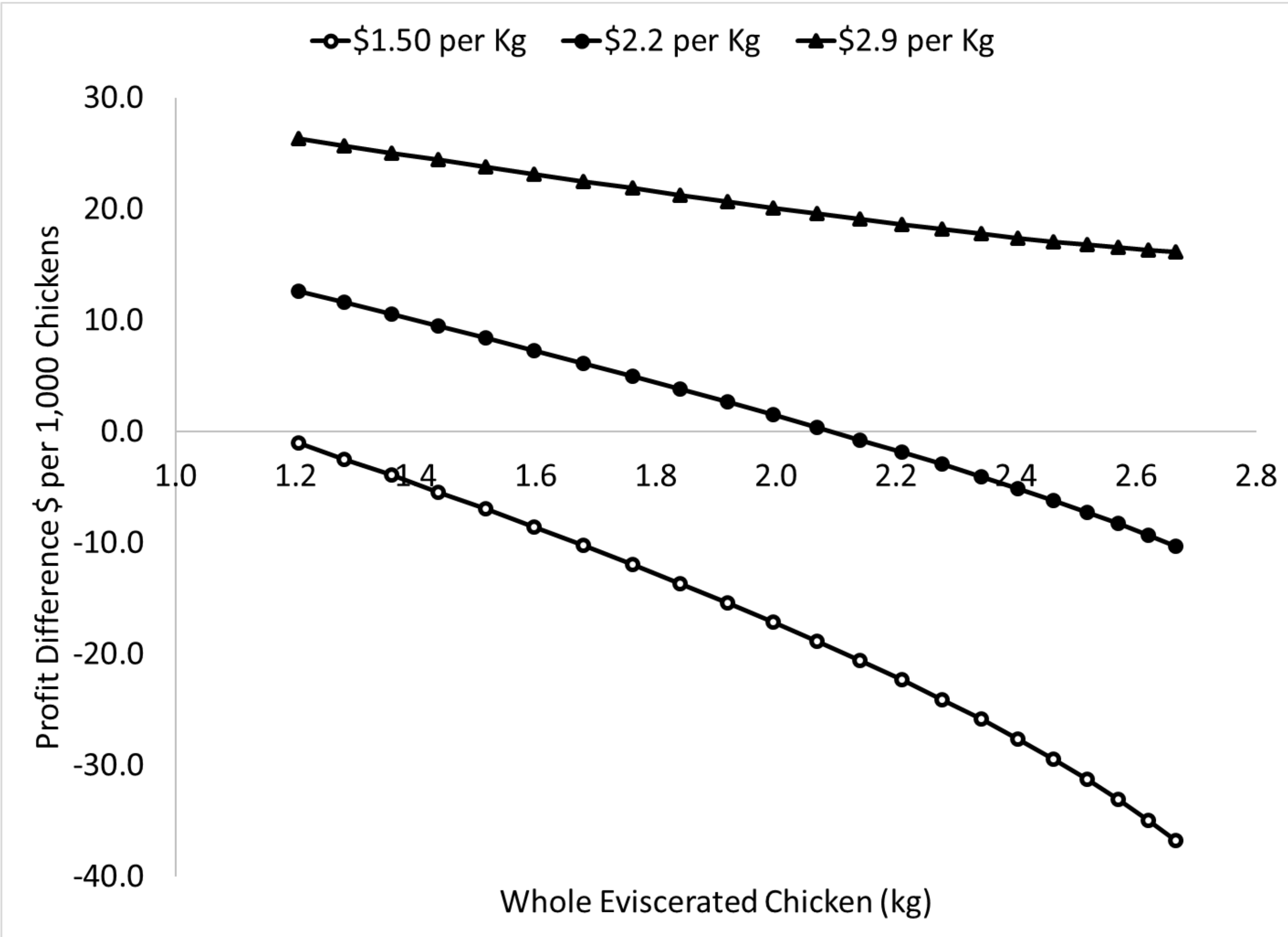

Figure 5.6. Difference in Returns Over Feed Cost (ROFC) at different sizes of chickens sold at three prices ($1.5, $2.2 or $2.9 per kg) from production of male broiler chickens fed diets formulated on the basis of Ajinomoto Heartland (AHI) database with an average formula cost of $539 per ton or Evonik Industries (ED) amino acid database with an average formula cost of $555 per ton (high feed costs). Profit difference was calculated as ROFC for ED minus ROFC for AHI times 1000. Positive difference indicates potential savings from using the ED database while negative values indicate savings from using the AHI database. All costs and prices are in US dollar.

CHAPTER 6

ESTIMATION OF THE MAXIMUM SAFE LEVEL OF FEED INGREDIENTS BY SPLINE OR BROKEN-LINE NONLINEAR REGRESSION MODELS [1]

[1] Alhotan, R. A., D. V. Vedenov and G. M. Pesti. Submitted to the Journal of Poultry Science, 06/24/16.

ABSTRACT

The use of non-linear regression models in the analysis of biological data has led to advances in poultry nutrition. Spline or broken-line nonlinear regression models are commonly used to estimate nutritional requirements. One particular application of broken-line models is estimating the maximum safe level (**MSL**) of feed ingredients beyond which the ingredients become toxic, resulting in reduced performance. The objectives of this study were to evaluate the effectiveness of broken- line models (broken-line linear or **BLL**; and broken-line quadratic or **BLQ**) in estimating the MSL; to identify the most efficient design of feeding trials by finding the optimal number of ingredient levels and replications; and to re-estimate the MSL of various test ingredients reported in the nutrition literature for comparison purposes. The Maximum Ingredient level Optimization Workbook (**MIOW**) was developed to simulate a series of experiments and estimate the MSL and the corresponding descriptive statistics (SD, SE, CI, and R^2). The results showed that the broken-line models provided good estimates of the MSL (small SE and high R^2) with the BLL model producing higher MSL values as compared to the BLQ model. Increasing the number of experimental replications or ingredient levels (independently of each other) reduced the SE of the MSL with diminishing returns. The SE of the MSL was reduced with increasing the size (total pens) of the simulated experiments by increasing either the number of replications or levels or both. The evaluation of MSLs reported in the existing literature revealed that the multiple range procedure used to determine the MSL in several reports can both overestimate and underestimate the MSL compared to the results obtained by the broken-line models. The results suggest that the broken-line linear models can be used in lieu of the multiple range test to estimate the MSL of feed ingredients along with the corresponding descriptive statistics, such as the SE of the MSL.

Key words: Feed ingredient, Safe level, Spline function, Broken-line

INTRODUCTION

Non-linear regression has been a useful tool in modeling the biological responses of poultry. Two of the most commonly used non-linear regression models are the broken-line linear (**BLL**) and the broken-line quadratic (**BLQ**) models. These models are a subset of spline models which are characterized by piecewise polynomials connected at the “spline knots”. Nutritionists have used the broken-line models to estimate the nutritional requirements of poultry (Robbins *et al*., 1979; Firman and Boling, 1998; Faria *et al*., 2002; Mehri *et al*., 2010). Vedenov and Pesti (2008) adapted Microsoft Excel to estimate the nutritional requirements using the broken-line models. The assumption underlying the broken-line models is that feeding increasing levels of a particular nutrient results in a change in the response (increase or decrease depending on the parameter measured) up to a certain point (minimum or maximum requirement) at which the response plateaus. The BLL model fits two linear segments of the biological response to a nutrient (the descending or ascending segment and the plateau). The BLQ combines a quadratic (descending or ascending) segment and a linear segment (plateau) and typically produces a wider confidence interval than the BLL model. Both BLL and BLQ models can also be used to estimate the maximum safe level (**MSL**) of new feed ingredients.

New feed ingredients (new processes, cultivates, etc.) are routinely introduced in the poultry industry to maximize the economic efficiency of production. Since feedstuffs vary in nutrient quality (e.g. fiber, anti-nutritional factors, AA profile, etc.), estimating the MSL of the ingredients is necessary to avoid any detrimental effects on biological performance. To estimate the MSL of a new ingredient, a dose-response study involving feeding birds increasing levels of the test ingredient has to be conducted. In the dose-response study, one or more measurements (e.g. BW gain, feed efficiency or breast meat yield) are taken for each growth phase and the

MSL of the test ingredient is determined for each measurement for that phase (starter, grower, etc.). Several statistical methods have been used to estimate the MSL including multiple-range tests (Hidalgo *et al*., 2004; Yamauchi *et al*., 2006; Aziza *et al*., 2010; Khempaka *et al*., 2013), orthogonal contrasts (Farran *et al*, 2000) and the regression approach using orthogonal polynomials (Proudfoot and Hulan, 1988; Kalmendal *et a*l., 2011; Gopinger *et al*. 2014). Multiple-range tests have been used extensively, mainly due to the ease of performing the tests. However, they have several critical shortcomings. First, multiple-range tests can only distinguish between the levels of the input factor. The levels of the test ingredient are treated as discrete, not continuous, which is a faulty assumption since the responses to the factors in the dose-response studies are continuous. Second, more conservative tests (Scheffe, 1953) result in fewer significant differences, while less conservative tests (e.g. Fisher's LSD test, 1935) are most likely to result in false differences. So called "Orthogonal contrasts" are used to compare levels against the control group. These tests are more precise than the multiple-range tests (fewer number of comparisons are made) but they are not really orthogonal (Lomax and Hahs-Vaughn, 2013) and distinguish only between levels as with the multiple-range tests. In regression analysis, the factors are treated as continuous and the maximum response level is defined by finding the root of the 1st derivative. However, this model does not fit the plateau segment of the response.

From a statistical standpoint, all three statistical methods typically used to define the MSL are not precise. Therefore, finding new methods to estimate the MSL of feed ingredients is necessary to maximize the economic efficiency of production. Theoretically, the response to feeding an ingredient with a limiting factor (e.g. toxic substance) can be modeled using the BLL and BLQ models such that the parameter signs (+, -, ≤, ≥) are changed to convert the functional forms to appear as mirror images of the original BLL and BLQ requirement models (Vedenov and Pesti, 2008). The requirement point then defines the MSL of the ingredient in the manner similar to how BLL and BLQ models are used to estimate the nutritional requirements. The NRC (1994) defined toxicity as "any adverse effect on performance". That broad definition is used here to illustrate any reduction in performance due to unfavorable nutrient composition. It could indicate pathological changes in the test birds but also any decreases in growth rate or feed intake. The objectives of the current research were (1) to evaluate the effectiveness of BLL and the BLQ models in estimating the MSL of feed ingredients using simulations in Microsoft Excel; (2) to examine the effect of the experiment design parameters (such as number of replications, ingredient levels and the number of simulations during the simulation process when planning feeding trials) on the estimated parameters and their descriptive statistics; and (3) to re-estimate the MSL values for broiler growth and egg production data obtained from the nutrition literature and compare them with the original MSL values reported by the authors. The Maximum Ingredient Level Optimization workbook or MIOW.xlsm was developed to achieve these objectives (Alhotan *et al.*, 2015).

MATERIALS AND METHODS

Development of the Maximum Ingredient Level Optimization Workbook

The Maximum Ingredient Level Optimization Workbook (MIOW.xlsm; Figure 6.1) guide and software are published elsewhere (Alhotan *et al.*, 2015; Appendix C). The Levels and Reps worksheet provides an option to create an experimental grid reflecting the desired levels of the test ingredient and the number of replications of the experiment. The Simulations worksheet allows to choose the "true" response model, displays the inputs for the true parameters, and allows input of initial guesses of coefficients for the regression models of interest (i.e. maximum response, rate constant and maximum ingredient level corresponding to the maximum response). The workbook then simulates experiment responses from the "true" model and fits desired regression models to the simulated data. The outputs of the simulation process are also displayed on Simulations worksheet. The outputs include estimates of the MSL by broken-line models and the descriptive statistics. (Standard deviation, SD; Standard Error, SE; 95% Confidence Interval, CI).

The SD is the simple standard deviations of estimates across different simulated experiments and was calculated from the equation $SD = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}\left(\beta_i - \bar{\beta}\right)^2}$ where β_i is the estimate of a specific parameter obtained from the ith simulated experiment. SEs are square roots of the diagonal elements of the matrix $\hat{\sigma}^2\,[Z'(b)\,Z(b)]^{-1}$, where $Z(B) = \left.\frac{\partial f\,(x,\beta)}{\partial\beta}\right|_b$; $\hat{\sigma}^2\,\frac{[y-f(x,\beta)]'[y-f(x,\beta)]}{N-K}$; $f(x,b)$ is the nonlinear function of the parameters estimated, x and y are the inputs and responses from a given experiment, respectively, N is the number of observations, and K is the number of parameters.

The mathematical models evaluated are:

Broken-line spline with descending linear segment model:

$$y = \begin{cases} Maximum\ Response, & if\ x < MSL \\ Maximum\ Response + Rate\ Constant \times (-Ingredient\ Level + x) & if x \geq MSL \end{cases} \quad (1)$$

Broken-line spline with descending quadratic segment model

$$y = \begin{cases} Maximum\ Response, & if x < MSL \\ Maximum\ Response + Rate\ Constant \times (Ingredient\ Level - x)^2 & if x \geq MSL \end{cases} \quad (2)$$

Here y is the response variable, x is the factor level and MSL is the maximum safe level.

The functions in (1) and (2) are modified versions of the broken-line functions used to estimate nutritional requirements (Vedenov and Pesti, 2008). This allows us to fit the plateau and descending segments to data from feeding trials in order to find the point at which the test ingredient becomes toxic.

Feeding Trial Planning

In order to identify the most efficient design for a feeding trial, growth data (16 to 24 days) from one reference using hulless barley (Anderson *et al*, 2012) were used to examine the effect of varying each of the following factors: (1) the number of simulated experiments (simulations); (2) the coefficient of variation (CV) of the of the simulated responses; (3) the number of replications of the ingredient levels; and (4) the number of ingredient levels. The optimal number of simulations was determined with CV fixed at 8%, number of ingredient levels at 4 and number of replications at 6. The CV was then varied from 0 to 32% for 100 simulations of 4 levels and 6 replications. The number of replications varied from 2 to 20 while fixing the number of ingredient levels at 4, number of simulations at 100, and CV at 8%. The number of levels was varied from 3 to 12 for 100 simulations with 6 replications and CV of 8%. Lastly, different combinations of levels and replications were tested by running 100 simulations each (CV=8%).

Re-estimation of Nutrition Literature Data

Broiler growth and egg production data from experiments conducted after the year 2000 to test the inclusion of various feed ingredients were compiled from the existing literature (Tables 6.1 and 6.2). The important criterion of selecting the data was that the response to a test ingredient declined after reaching a certain level (i.e. toxicity), so that the broken-line models can fit the plateau and descending segments. The final BWG and egg production rate reported for each experiment (data set) were used as the response variable. The MIOW.xlsm workbook was used to simulated experimental data based on each data set with the actual number of ingredient levels, number of replications, minimum and maximum ingredient levels, maximum growth and the corresponding ingredient level reported in the original publication. The corresponding CVs were calculated from the reported standard errors. The experiment data was generated using the BLQ model as the "true" model for all simulations. A total of 100 simulations were generated for each data set. The MSL estimated from the simulated experiments were averaged as the MSL mean and the descriptive statistics. (SD, SE, and 95% CI).

RESULTS

Feeding Trial Planning

The baseline SD of the MSL for 10 simulations repeated 5 times was estimated to be ~ 0.065 and 0.141 for the BLL and BLQ models, respectively (Table 6.3). Increasing the number of simulations from 10 to 100 reduced the SD of the MSL by at least 60% for the BLL and BLQ models. Increasing the number of simulations beyond 100 simulations resulted in further reductions in the SD of the MSL. Table 6.4 demonstrates that as the CV of the simulations increased from 0 to 32%, the SD and SE of the MSL estimates increased gradually and the R^2 values decreased for both models. Regardless of the model used, using more replications at a fixed number of levels (N=4) reduced the SD and SE of the MSL with diminishing returns (Table 6.5). As the number of ingredient levels increased, fixing the number of replications at 6, the SE of the MSL decreased for both models with diminishing returns (Table 6.6). Figure 6.2 depicts the distribution of data (Khempaka et al, 2013) when the CV, number of levels, number of replications is doubled when using the MIOW workbook to estimate the MSL. When the size of the simulated experiment was increased from 24 to 36 pens the SE of the MSL (SE average of all “24 pens” vs. all “36 pens”) was reduced by 14% (0.29 vs. 0.25) for the BLL model and 21% (0.57 vs. 0.45) for the BLQ model (Table 6.7). Further reduction in the SE by about 13% for both models was observed when the size was increased from 36 to 48 pens (SE average of all “36 pens” vs. all “48 pens”). Given the same resources (e.g. 24 pens), using more replications than levels (e.g. a combination of 6 reps x 4 levels vs. 4 reps x 6 levels) decreased the SE of the MSL for the BLL model. For the BLQ model, on the contrary, using more levels than reps reduced the SE.

Re-estimation of Nutrition Literature Data

The simulation results for broiler growth data in Table 6.8 showed that the recalculated MSL ranged between 4.16% ± 0.03 to 73.33% ± 0.12 for the BLL model and from 2.49% ± 0.06 to 59.99% ± 0.26 for the BLQ model. The R^2 values of the fitted relationships for both models ranged between ~ 0.93 to 1.0. The recalculated MSL for egg production data (Table 6.9) ranged between 12.57% ± 0.16 to 46.33% ± 0.06 and 9.28% ± 1.19 to 45.51% ± 0.66 for the BLL and BLQ models, respectively. The R^2 values were in the range 0.74 to 1.0 for both models. The recalculated MSL values by the BLQ model for all data sets for growth and egg production data were always smaller than those values obtained by the BLL model. On average, the BLQ values are 16.6% smaller than the BLL values as represented by the slope of the regression line ($MSL_{BLQ} = f(MSL_{BLL}$; Figure 6.3). For growth data (Table 6.8), the multiple range procedure overestimated the MSL for 6 data sets (4, 6, 7, 11, 12 and 13) and produced close estimates in 8 data sets (1, 2, 3, 5, 8, 9, 10 and 14) compared to BLQ estimates. Compared to the BLL estimates, the multiple range procedure overestimated the MSL for 6 data sets (4, 6, 7, 11, 12 and 13), underestimated the MSL for 3 data sets (2, 8 and 10) and produced close estimates for the remaining datasets (1, 3, 5, 9 and 14). For egg production data (Table 6.9), the multiple range procedure underestimated the MSL in data sets 4 and 8, overestimated the MSL in data sets 2 and 6 and resulted in close estimates in 4 data sets (1, 3, 5 and 7) compared to the BLQ model estimates. The MSL was underestimated by the multiple range in 4 data sets (3, 4, 5 and 8) and overestimated in 2 data sets (2 and 6) when compared to the BLL model.

DISCUSSION

Feeding Trial Planning

The MIOW workbook provides estimates of the MSL of feed ingredients based on a series of simulated experiments drawn from an assumed “true” model. The simulation outputs seem to be influenced not only by the true parameters but also by the initial guesses of coefficients used to estimated alternative model. Therefore, providing good initial guesses is necessary for more accurate results. The main purpose of the MIOW Workbook is to provide estimates of the MSL of feed ingredients and the corresponding descriptive statistics for planning new experiments. The simulation estimates of the MSL varied depending on the mathematical model (BLL or BLQ) being used. The MSL estimates for the BLL model are almost always greater than the BLQ model. Differences in the MSL estimates should be taken into consideration when deciding to estimate the MSL of a feed ingredient using the broken-line approach. Since the estimation of the MSL is based on the simulation process, providing a sufficient number of simulated experiments with some degree of variability is required. Increasing the number of simulated experiments from 10 to 100 decreased the SD of the MSL by ~ 60% for both models making the estimates more stable. The simulation process is based on drawing random numbers from the normal distribution, so the larger the sample size the more the random numbers are centered around the mean. A minimum of 50 simulated experiments seemed to be sufficient to produce satisfactory results (more stable estimates). Using a larger number of simulations is preferred but it can be time consuming. When the CV of simulations increased from 0 to 32%, the SE of the MSL estimated by BLL and BLQ models increased resulting in wider confidence intervals. The SE is particularly important because it indicates how accurate the MSL mean was and smaller values are always desirable. The increase in the SE was associated with a reduction in the R^2 implying poorer model fitting at high CV values.

The MIOW Workbook can particularly be useful to find the best combination of levels and replications when designing feeding trials and justifying numbers of birds or animals that need to be used. Increasing the number of replications per level from 2 to 4 reduced the SE of the MSL for both models by at least 13% and further increase from 4 to 6 replicates reduced the SE by at least 12%. Increasing the number of replications beyond 6 resulted in further reduction in the SE but with diminishing returns. This result demonstrates the importance of using the right number of replications when designing feeding trials for more precise MSL estimates. The impact of increasing the number of ingredient levels on the SE were very similar. Regardless of the model used, using 4 levels instead of 3 reduced the SE of the MSL by at least 15%. Further increase in the number of levels decreased the SE of the MSL, although again with diminishing returns. The SD of the MSL from the simulated experiments decreased with increasing the number of replications but increased with increasing the number of levels for both models. It should be noted that the SE and SD do not have to be in any particular relationship to each other. The extreme difference between the estimates of the MSL by BLL and BLQ (Table 6.6) is due to nature of models. Considering the available resources (e.g. space, amount of test ingredient, etc.), the best combination of levels and replications should have the smallest SE of the MSL given available resources. For instance, a feeding trial design of 6 levels of 8 replications each (a total of 48 pens) should be more efficient than a design of 6 levels of 4 replications (a total of 24 pens). MIOW workbook can quantify the increase in efficiency so that an informed decision can be made.

Re-estimation of Nutrition Literature Data

The broken-line models assume that feeding an ingredient with a limiting factor produces a response that declines when the toxicity level is reached. Therefore, biological responses that plateau and decline thereafter can only be fitted with these models. Several types of the response to test ingredients were encountered when searching for the optimal data to fit to the broken-line models. Linear increase in response, linear decrease in response, and increase in response followed by a plateau were among those types of responses. Moreover, quadratic increase and then decrease in response and data with the points scattered in the plane were also encountered. A linear decrease in response suggests that the ingredient is very toxic and low test levels are needed. The case were the data points are scattered in the plane suggests that the levels used are not high enough to show toxicity effects. Those types of responses demonstrate the importance of choosing the right ingredient levels to be tested and the importance of having preliminary data on expected safe levels and variation between observations. The multiple-range tests used in the studies listed (Tables 6.1 & 6.2; Figure 6.4) either gave close values, overestimated or underestimated the MSL of the ingredients compared to the MSL values obtained from the broken-line models. The multiple-range tests are appropriate for discrete variables but not continuous variables and interpretations are subject to the power of the statistical tests in detecting significant differences. In one study (Loar II *et al.*, 2012; Figure 6.5), the claimed MSL of DDGS to be included in broiler diets without any adverse effects on growth performance and carcass yield was 14% based on Fisher's LSD. Since the level 21% is not significantly different than the control group, the MSL could also be at or above this level (between 21 to 28%). Therefore, 21% DDGS could be considered to be a safe level to achieve a satisfactory weight gain during 0 - 42 d based on the LSD test (Figure 6.5). Using the BLQ model (Figure 6.5), the MSL was estimated to be 7% ± 0.75. The predicted gain at 7% DDGS was 2.69 kg and the gain when feeding the 21% diet was 2.59 kg (~ 100 g difference). Using the BLQ method in this example showed the MSL capturing the response before the point where toxicity began. Failure to estimate the MSL of an ingredient accurately could result in significant losses in performance due to high intake of anti-nutritional substances. Unlike the multiple-range tests, the broken-line approach offers estimations of several descriptive statistics of the MSL, such as the confidence interval, SE and SD, which provide important information about the accuracy of the estimates. Choosing one model over the other is practically impossible because the R^2 values of the simulated growth and egg production studies for both models are very similar.

In conclusion, the broken-line models of the MIOW workbook provide a tool to estimate the MSL of feed ingredients and to find the most efficient design of feeding trials by simulating a series of experiments. The most efficient design should be the combination of ingredient levels and replications that produces the smallest SE of the MSL. Compared to the broken-line estimates, the MSL of the data evaluated was either overestimated or underestimated by the multiple range test in several reports. Unlike the multiple range procedure, the broken-line models treat the levels of the ingredient as continuous and provide several descriptive statistics of the MSL.

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Table 6.1. Feed ingredient levels and the mean separation method used in feeding trials of broilers experiments compiled from the nutrition literature

Reference	Test Ingredient	Levels Used (%)	Means Separation Method	Pattern of Response [2]		
				Linear	Quadratic	Cubic
Gamboa *et al*, 2001	Cottonseed meal	0, 7, 14, 21, 28	Tukey's test	-	-	-
Newkirk & Classen, 2002	Canola meal	0, 20, 40, 60, 80, 100	Duncan's multiple-range test	√	√	√
Lee *et al*, 2003	Guar meal	0, 2.5, 5, 7.5, 10	Duncan's multiple-range test	-	-	-
Gallinger *et al*, 2004	Rice bran	0, 10, 20, 30, 40	Duncan's multiple-range test	-	-	-
Lumpkins *et al*, 2004	DDGS[1]	0,6,12,18	Fisher's LSD test	-	-	-
Khempaka *et al*, 2009	Cassava pulp	0, 4, 8, 12, 16	Fisher's LSD test	√	√	√
Loar II *et al*, 2010	DDGS[1]	0, 7.5, 15, 22.5, 30	Fisher's LSD test	√	√	-
Jung and Batal, 2011	Crude glycerin	Variable	Duncan's multiple-range test	-	-	-
Anderson *et al*, 2012	Hulless barley	0, 15, 30, 45	Tukey-Kramer test	-	-	-
Loar II *et al*, 2012	DDGS[1]	0, 7, 14, 21, 28	Fisher's LSD test	√	√	-
Supriyati *et al*, 2015	Rice bran	0, 5, 10, 15, 20	Duncan's multiple-range test	√	√	-
Evans *et al*, 2015	Algae	0, 6, 11, 16, 21	Fisher's LSD test	-	-	-
Campasino *et al*, 2015	DDGS[1]	0, 5, 10, 15	Duncan's multiple-range test	-	-	-

[1] Distillers dried grains with solubles.
[2] Linear, quadratic and cubic contrasts to examine the pattern or response by original authors. A Check mark indicates the contrast was tested for while a dash indicates the that the contrast was not tested for by original authors.

Table 6.2. Feed ingredient levels and the mean separation method used in feeding trials of laying hens experiments compiled from the nutrition literature

Reference	Test Ingredient	Levels Used (%)	Means Separation Method	Pattern of Response [2]		
				Linear	Quadratic	Cubic
Perez *et al*, 2000	Palm kernel meal	0, 10, 20, 30, 40, 50	Fisher's LSD test	-	-	-
Braga *et al*, 2005	Coconut meal	0, 5, 10, 15, 20	Dunnett's test	-	-	-
Cherian *et al*, 2009	Camelina sativa	0, 5, 10, 15	Duncan's multiple-range test	-	-	-
Al-kirshi *et al*, 2010	Mulbery leaf meal	0,10, 15, 20	Duncan's multiple-range test	-	-	-
Mirzaie *et al*, 2012	Wheat	0, 23, 46, 69	Tukey's test	√	√	-
Sun *et al*, 2012	DDGS[1]	0, 17, 35, 50	Duncan's multiple-range test	-	-	-
Deniz *et al*, 2013	DDGS[1]	0, 5, 10, 15, 20	Tukey's test	-	-	-
Araújo *et al*, 2015	Sunflower Meal	0, 8, 16, 24	Student-Newmann-Keul test	-	-	-

[1] Distillers dried grains with solubles.
[2] Linear, quadratic and cubic contrasts to examine the pattern or response by original authors. A Check mark indicates the contrast was tested for while a dash indicates the that the contrast was not tested for by original authors.

Table 6.3. Effect of increasing the number of simulated experiments on the standard deviation of the maximum safe level (MSL) of whole hulless barley estimated by two broken line models with linear (BLL) and quadratic (BLQ) descending segments (CV= 8%; level= 4; reps= 6)

Number of Simulations	MSL (BLL Model)	± SD[1]	MSL (BLQ Model)	± SD[1]
	11.900	0.160	6.106	0.353
	11.816	0.179	5.912	0.387
10	11.983	0.125	6.268	0.289
	11.952	0.072	6.190	0.185
	11.876	0.212	6.013	0.498
SD[2]	0.065		0.141	
	11.875	0.153	6.035	0.351
	11.829	0.177	5.918	0.402
50	11.837	0.169	5.931	0.369
	11.859	0.154	5.978	0.358
	11.873	0.160	6.025	0.371
SD[2]	0.021		0.053	
% Δ[3]	-68.207		-62.331	
	11.868	0.173	6.011	0.381
	11.889	0.149	6.056	0.342
100	11.860	0.194	6.000	0.430
	11.841	0.162	5.947	0.356
	11.830	0.154	5.910	0.351
SD[2]	0.023		0.057	
% Δ[3]	12.422		7.107	
	11.869	0.157	6.008	0.355
	11.849	0.165	5.961	0.378
500	11.861	0.163	5.990	0.373
	11.866	0.161	6.001	0.367
	11.860	0.162	5.990	0.369
SD[2]	0.008		0.018	
% Δ[3]	-67.181		-67.932	
	11.860	0.158	5.990	0.361
	11.861	0.162	5.995	0.370
1000	11.861	0.161	5.987	0.373
	11.862	0.165	5.991	0.380
	11.860	0.160	5.989	0.367
SD[2]	0.001		0.003	
% Δ[3]	-91.052		-83.929	

[1] Standard deviation within runs (optimizations)
[2] Standard deviation between runs (optimizations)
[3] Percent change in SD as compared to the previous value

Table 6.4. Effect of increasing variation of the simulated experiments on estimating the maximum safe level (MSL) of whole hulless barley by two broken-line models with linear (BLL) and quadratic (BLQ) descending segments (simulations = 100; level= 4; reps= 6)

	BLL Model							BLQ Model						
CV (%)[1]	Max (g)[2]	MSL (%)	± SD	± SE	95% CI Lower	95% CI Upper	R^2	Max (g)[2]	MSL (%)	± SD	± SE	95% CI Lower	95% CI Upper	R^2
0	54.14	12.58	0.00	0.01	12.58	12.58	1.00	56.10	6.00	0.00	0.00	6.00	6.00	1.00
2	54.10	12.58	0.03	0.09	12.57	12.58	1.00	56.06	5.99	0.07	0.25	5.98	6.01	1.00
4	54.08	12.57	0.05	0.17	12.56	12.58	1.00	56.09	5.97	0.14	0.46	5.95	6.00	1.00
8	54.21	12.58	0.11	0.35	12.56	12.60	0.99	56.16	6.01	0.30	0.95	5.95	6.07	0.99
14	54.03	12.54	0.19	0.62	12.51	12.58	0.98	56.19	5.91	0.50	1.69	5.81	6.01	0.98
16	53.68	12.61	0.22	0.64	12.57	12.66	0.98	55.51	6.10	0.58	1.77	5.99	6.21	0.98
32	54.12	12.59	0.42	1.34	12.51	12.67	0.91	56.23	6.07	1.09	3.66	5.86	6.28	0.91

[1] Coefficient of Variation
[2] Estimated maximum daily gain in grams

Table 6.5. Effect of increasing the number of replications on estimating the maximum safe level (MSL) of whole hulless barley by two broken-line models with linear (BLL) and quadratic (BLQ) descending segments (simulations= 100; CV= 8%; level= 4)

	BLL Model						BLQ Model					
					95% CI						95% CI	
N[1]	Max (g)[2]	MSL (%)	± SD	± SE	Lower	Upper	Max (g)[2]	MSL (%)	± SD	± SE	Lower	Upper
2	53.03	12.54	0.33	0.78	12.47	12.60	55.25	5.91	0.88	2.14	5.74	6.09
4	54.24	12.56	0.23	0.68	12.51	12.60	56.40	5.95	0.61	1.85	5.83	6.07
6	53.85	12.59	0.19	0.59	12.56	12.63	55.77	6.05	0.49	1.63	5.95	6.15
8	54.65	12.56	0.15	0.52	12.53	12.59	56.79	5.96	0.42	1.43	5.87	6.04
12	54.09	12.58	0.13	0.45	12.55	12.61	56.09	6.00	0.36	1.23	5.93	6.07
16	54.28	12.55	0.11	0.40	12.53	12.57	56.46	5.91	0.29	1.09	5.86	5.97
20	54.10	12.58	0.10	0.34	12.56	12.60	56.05	6.01	0.28	0.94	5.96	6.07

[1] Number of replications
[2] Estimated maximum daily gain in grams

Table 6.6. Effect of increasing the number of levels on estimating the maximum safe level (MSL) of whole hulless barley by two broken-line models with linear (BLL) and quadratic (BLQ) descending segments (simulations= 100; CV= 8%; reps= 6)

	BLL Model						BLQ Model					
					95% CI						95% CI	
N[1]	Max (g)[2]	MSL (%)	± SD	± SE	Lower	Upper	Max (g)[2]	MSL (%)	± SD	± SE	Lower	Upper
3	55.93	9.99	0.07	0.30	9.97	10.00	55.93	6.01	0.18	0.83	5.97	6.04
4	54.20	11.86	0.16	0.24	11.83	11.89	56.19	5.99	0.35	0.58	5.92	6.06
5	54.14	10.78	0.91	0.34	10.60	10.96	55.81	6.05	0.38	0.44	5.98	6.13
6	50.79	11.57	0.15	0.26	11.54	11.60	55.96	6.02	0.40	0.42	5.94	6.10
8	50.14	11.42	0.13	0.23	11.39	11.45	56.07	5.98	0.37	0.34	5.90	6.05
10	49.69	11.35	0.13	0.21	11.32	11.37	56.05	5.97	0.37	0.30	5.90	6.04
12	49.46	11.32	0.09	0.19	11.30	11.34	56.11	5.98	0.28	0.27	5.93	6.04

[1] Number of ingredient levels
[2] Estimated maximum daily gain in grams

Table 6.7. Effect of different combinations of levels and replications on estimating the maximum safe level (MSL) of whole hulless barley by two broken-line models with linear (BLL) and quadratic (BLQ) descending segments (simulations= 100; CV= 8%)

Pens	Levels	Reps	BLL Model						BLQ Model					
			Max (g)[1]	MSL (%)	± SD	± SE	95% CI		Max (g)[1]	MSL (%)	± SD	± SE	95% CI	
							Lower	Upper					Lower	Upper
24	3	8	56.14	9.98	0.05	0.26	9.97	9.99	56.14	6.00	0.14	0.72	5.97	6.02
24	8	3	50.28	11.43	0.18	0.32	11.39	11.46	56.12	6.01	0.49	0.45	5.92	6.11
24	4	6	53.95	11.87	0.15	0.25	11.84	11.90	55.99	5.99	0.34	0.59	5.93	6.06
24	6	4	50.64	11.59	0.18	0.31	11.55	11.62	55.67	6.07	0.48	0.49	5.98	6.17
36	3	12	55.98	9.98	0.05	0.21	9.97	9.99	55.98	6.00	0.13	0.58	5.97	6.02
36	12	3	49.53	11.32	0.14	0.27	11.29	11.35	56.18	5.99	0.39	0.38	5.91	6.06
36	4	9	53.94	11.86	0.13	0.21	11.84	11.89	55.95	5.99	0.29	0.49	5.93	6.04
36	9	4	52.72	10.91	0.44	0.29	10.83	11.00	55.86	6.08	0.44	0.38	6.00	6.17
36	6	6	50.77	11.57	0.14	0.25	11.54	11.60	55.84	6.04	0.38	0.41	5.96	6.11
48	4	12	54.26	11.87	0.13	0.18	11.84	11.89	56.20	6.01	0.30	0.42	5.95	6.07
48	12	4	49.49	11.32	0.12	0.24	11.30	11.35	56.08	6.00	0.35	0.33	5.93	6.07
48	6	8	50.93	11.56	0.12	0.22	11.54	11.58	56.11	6.00	0.31	0.36	5.94	6.06
48	8	6	50.28	11.42	0.14	0.23	11.40	11.45	56.21	5.98	0.39	0.35	5.91	6.06
48	4	12	54.18	11.88	0.12	0.17	11.86	11.90	56.15	6.02	0.28	0.41	5.97	6.08
48	12	4	49.65	11.32	0.13	0.24	11.30	11.35	56.22	6.00	0.37	0.33	5.93	6.08

[1] Estimated maximum daily gain in grams

Table 6.8. Reported and re-estimated maximum safe level (MSL) of feed ingredients for broilers experiments compiled from the nutrition literature

NO	Reference	Reported MSL (%)[1]	Broken-line with descending linear segment					Broken-line with descending quadratic segment				
			MSL[2]	± SD	95% CI		R^2	MSL[2]	± SD	95% CI		R^2
					Lower	Upper				Lower	Upper	
1	Gamboa *et al*, 2001	21.0	21.08	0.03	21.08	21.09	0.988	20.92	0.14	20.90	20.95	0.988
2	Newkirk & Classen, 2002	60.0	73.33	0.12	73.31	73.35	0.999	59.99	0.26	59.94	60.04	0.999
3	Lee *et al*, 2003	7.5	7.76	0.09	7.74	7.78	0.978	7.48	0.08	7.46	7.50	0.978
4	Gallinger *et al*, 2004	20.0	18.30	0.19	18.26	18.34	0.979	9.92	0.72	9.78	10.06	0.990
5	Lumpkins *et al*, 2004	12.0	12.04	0.01	12.03	12.04	1.000	11.96	0.05	11.95	11.97	1.000
6	Khempaka *et al*, 2009	8.0	7.31	0.27	7.26	7.37	0.980	3.88	0.54	3.77	3.99	0.990
7	Loar II *et al*, 2010	15.0	13.73	0.10	13.71	13.75	0.985	7.44	0.36	7.37	7.51	0.996
8	Jung and Batal, 2011	2.5	4.16	0.03	4.15	4.17	0.996	2.49	0.06	2.47	2.50	0.996
9	Jung and Batal, 2011	5.0	5.06	0.06	5.04	5.07	0.970	5.00	0.09	4.98	5.02	0.970
10	Anderson *et al*, 2012	15.0	25.04	0.34	24.97	25.10	0.992	15.08	0.78	14.93	15.24	0.992
11	Loar II *et al*, 2012	21.0	12.84	0.10	12.82	12.86	0.983	7.01	0.38	6.94	7.09	0.995
12	Supriyati *et al*, 2014	15.0	9.27	0.76	9.12	9.41	0.932	5.05	1.22	4.81	5.29	0.943
13	Evans *et al*, 2015	16.0	14.33	0.04	14.32	14.33	0.997	10.99	0.08	10.97	11.00	0.997
14	Campasino *et al*, 2015	10.0	10.03	0.01	10.03	10.03	0.998	9.97	0.04	9.96	9.98	0.998

[1] The reported maximum safe level of the test ingredient by the original authors for a satisfactory body weight gain.
[2] The re-estimated maximum safe level of the test ingredient by the current models for a satisfactory body weight gain

Table 6.9. Reported and re-estimated maximum safe level (MSL) of feed ingredients for laying hens experiments compiled from the nutrition literature

No	Reference	Reported MSL (%)[1]	Broken-line with descending linear segment					Broken-line with descending quadratic segment				
			MSL[2]	± SD	95% CI		R^2	MSL[2]	± SD	95% CI		R^2
					Lower	Upper				Lower	Upper	
1	Perez *et al*, 2000	40.0	40.26	0.53	40.15	40.36	0.903	39.02	1.43	38.74	39.30	0.903
2	Braga *et al*,2005	20.0	13.33	0.03	13.33	13.34	0.999	10.00	0.07	9.99	10.01	0.999
3	Cherian *et al*,2009	10.0	12.57	0.16	12.54	12.60	0.742	9.28	1.19	9.04	9.51	0.745
4	Al-kirshi *et al*, 2010	10.0	16.87	0.23	16.82	16.91	0.961	14.48	0.71	14.35	14.62	0.962
5	Deniz *et al*, 2012	15.0	16.90	0.02	16.90	16.91	0.998	14.73	0.31	14.66	14.79	0.998
6	Mirzaie *et al*, 2012	69.0	46.33	0.06	46.32	46.34	0.997	45.51	0.66	45.39	45.64	0.997
7	Sun *et al*, 2012	35.0	36.01	0.13	35.99	36.04	0.993	34.54	0.61	34.42	34.66	0.993
8	Araújo *et al*, 2015	6.72	17.67	0.08	17.65	17.68	0.993	15.56	0.64	15.44	15.68	0.993

[1] The reported maximum safe level of the test ingredient by the original authors for a satisfactory egg production rate
[2] The re-estimated maximum safe level of the test ingredient by current models for a satisfactory egg production rate

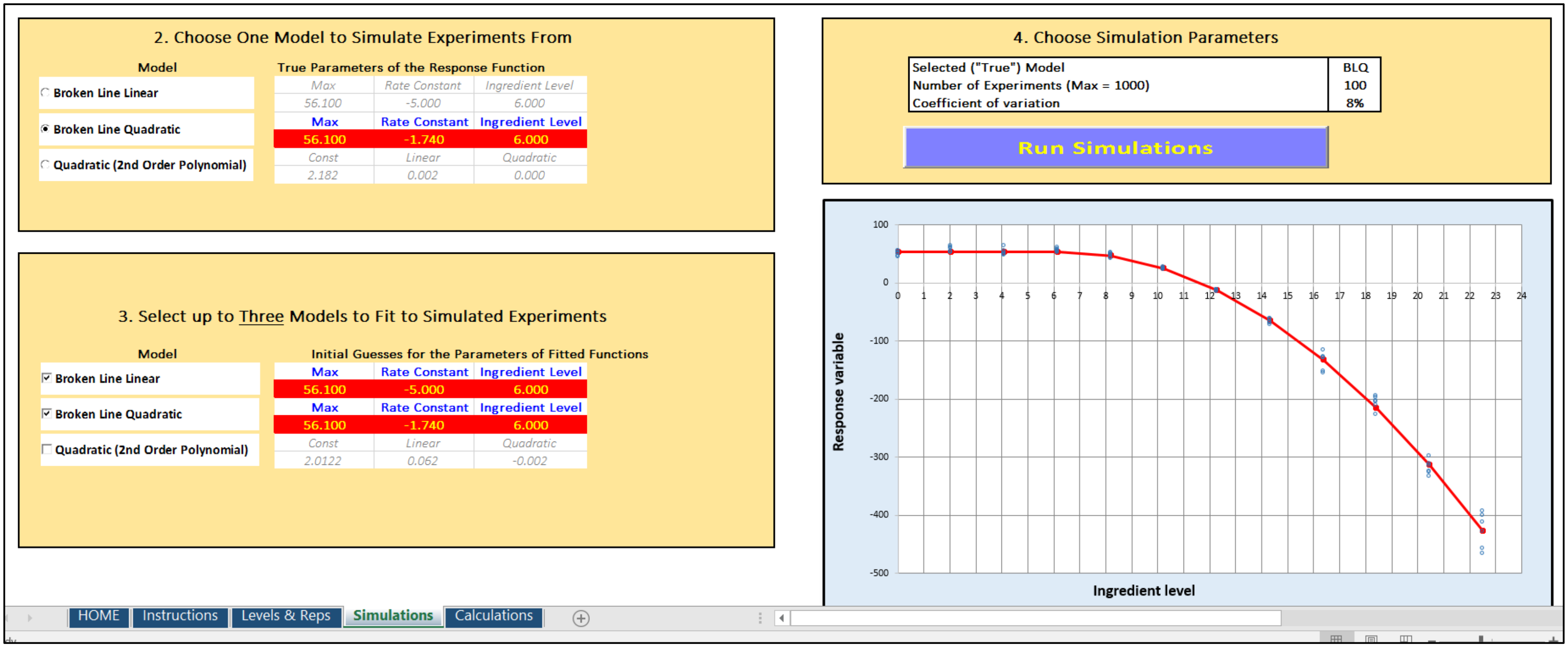

Figure 6.1. Overview of the major spreadsheet "Simulations" of the Maximum Ingredient Optimization Workbook (MIOW)

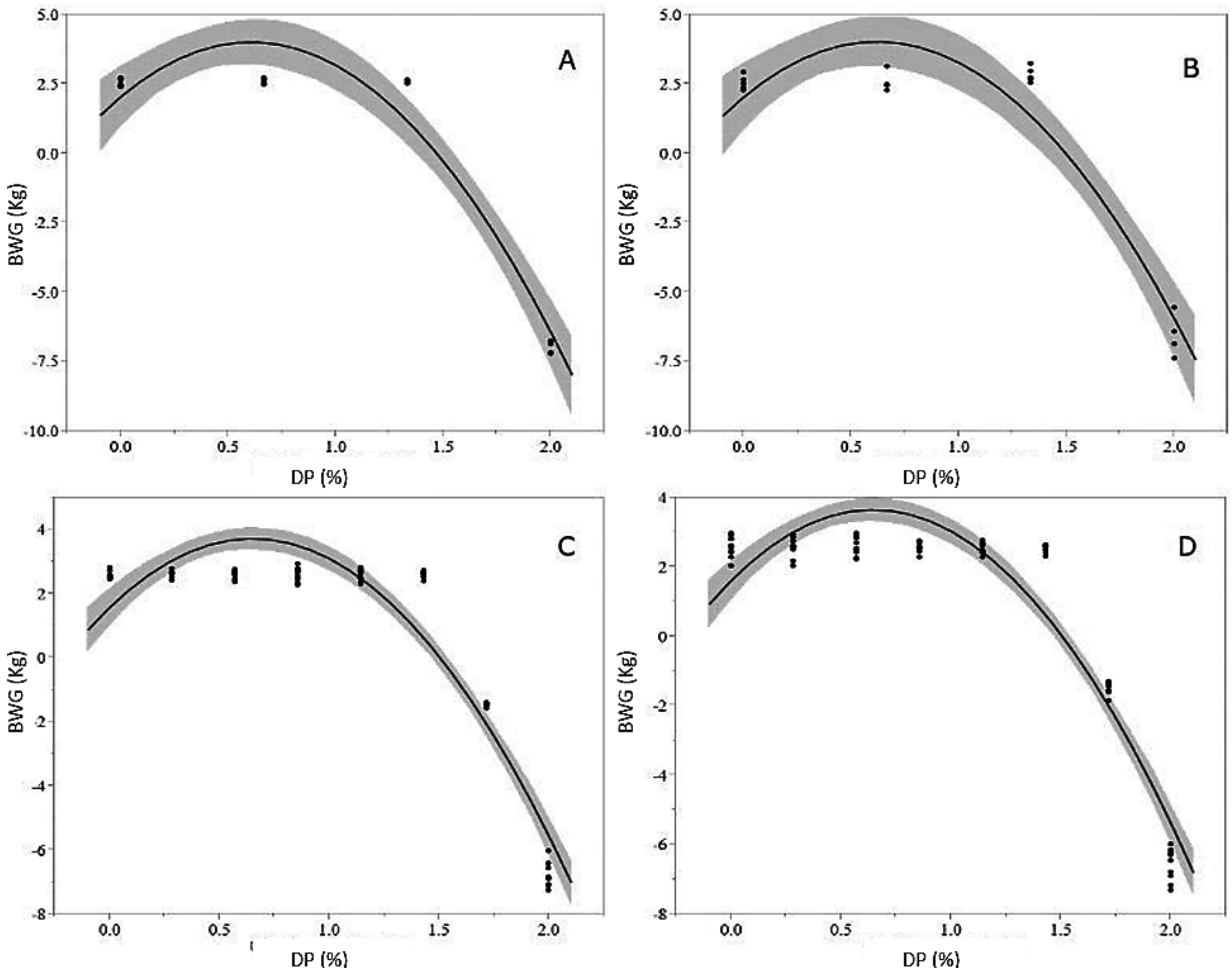

Figure 6.2. Simulations of the growth response of broiler chickens to increasing levels of dried Peppermint (DP; Khempaka *et al*, 2013). (A) Based on 100 simulated experiments (CV=5%) of 4 levels and 5 replications. (B) Based on 100 simulated experiments (CV=10%) of 4 levels and 5 replications. (C) Based on 100 simulated experiments (CV=5%) of 8 levels and 10 replications. (D) Based on 100 simulated experiments (CV=10%) of 8 levels and 10 replications.

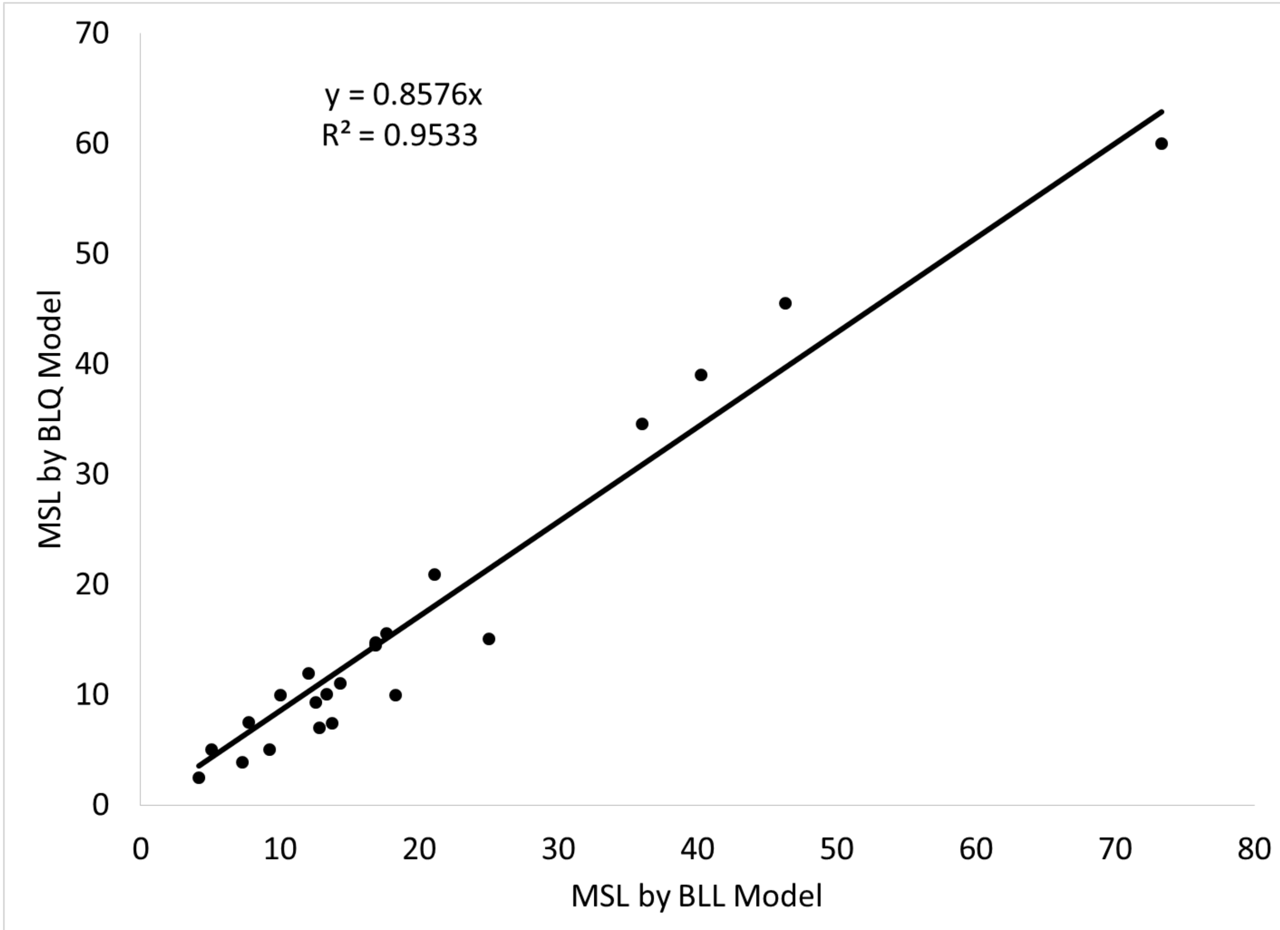

Figure 6.3. The relationship between the re-estimated MSL values by broken-line models with linear (BLL) and quadratic (BLQ) descending segments for layers and broilers. The relationship between y (BLQ) and x (BLL) as defined by the equation y=0.8576x is 1/0.8576 =1.166. Therefore, the difference between y and x is 0.166 or 16.6%.

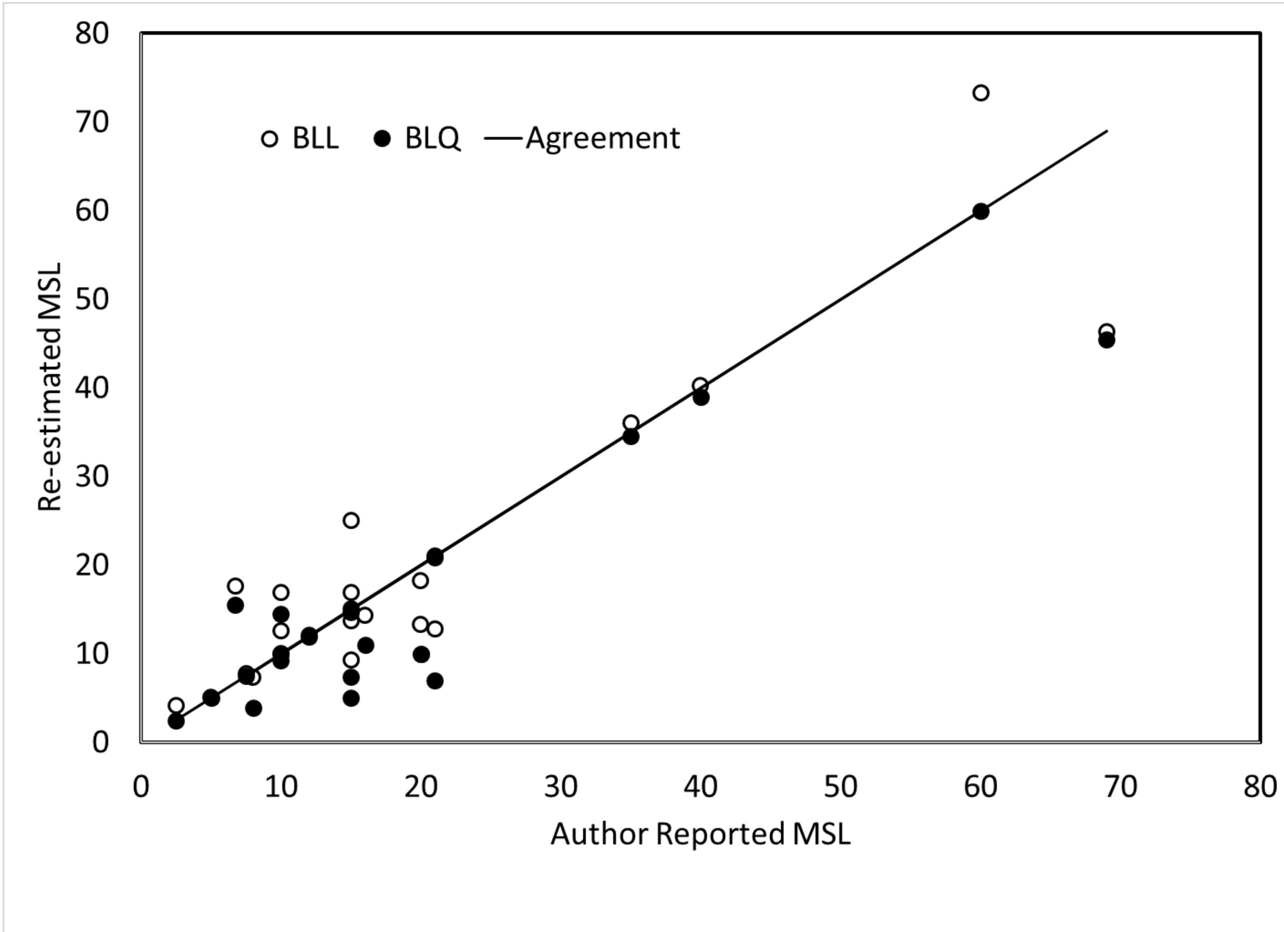

Figure 6.4. The relationship between the re-estimated maximum safe level (MSL) values by broken-line models with linear (BLL) and quadratic (BLQ) descending segments and the author reported ones for layers and broilers. The “Agreement” line represents perfect agreement (slope =1) between author reported (based on multiple range tests) and estimates based on BLL and BLQ models.

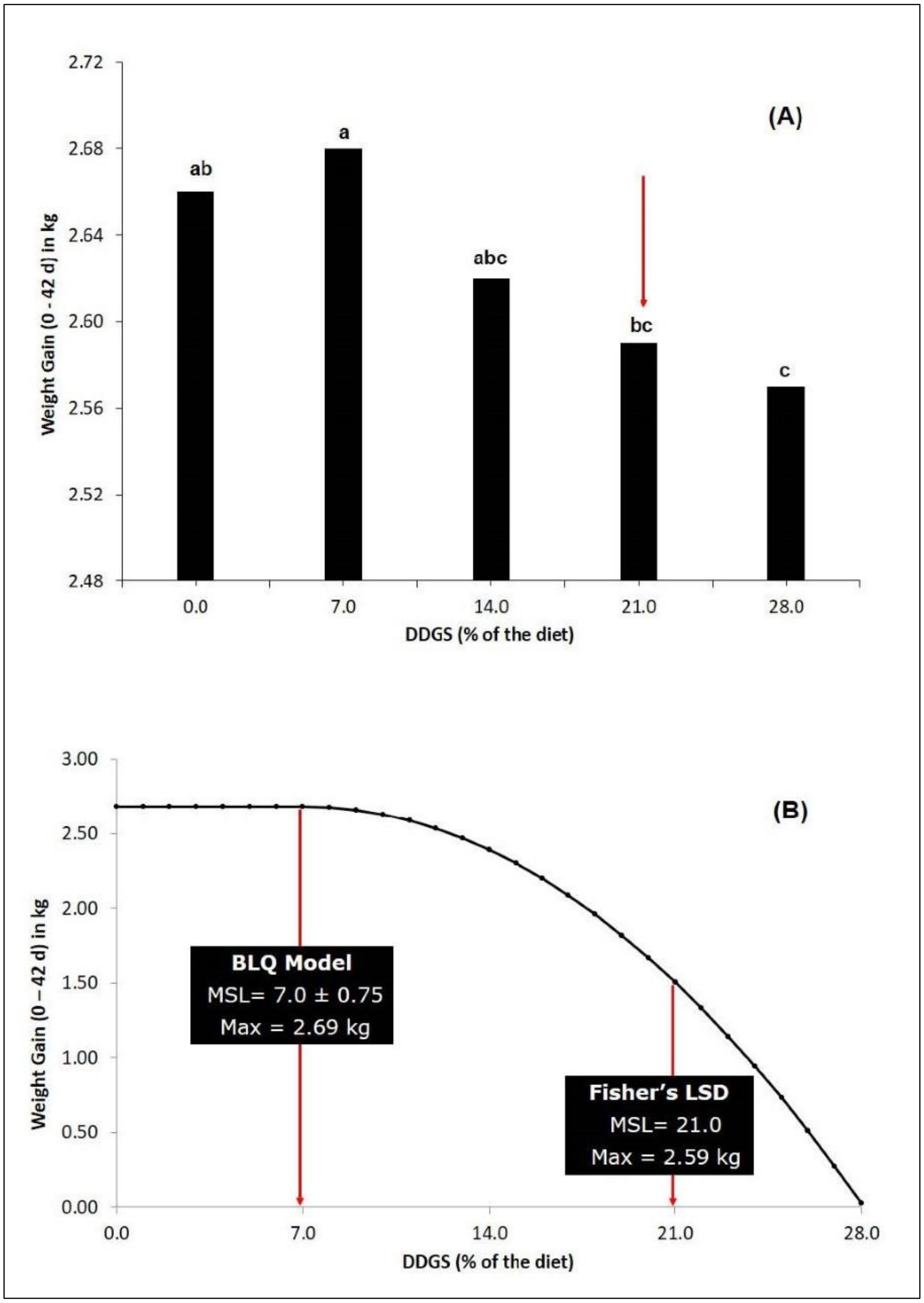

Figure 6.5. The maximum safe level (MSL) of DDGS as estimated by (A) Fisher's LSD test and (B) broken-line model with quadratic (BLQ) descending segment. Note that 21% DDGS in (A) gave equivalent response to the control ($P > 0.05$) so the 21% DDGS should be considered safe (although the authors concluded that the MSL=14%). The estimate of MSL for DDGS by the BLL model (Table 6.8) is 12.84 ± 0.10 which is close to the claimed MSL but less than 21%.

CHAPTER 7

NUTRITIVE VALUE AND THE MAXIMUM INCLUSION LEVEL OF PENNYCRESS AND CANOLA MEALS FOR BROILER CHICKENS [1]

[1] Alhotan, R. A., R. Holser and G. M. Pesti. To be submitted to the Journal of Poultry Science

ABSTRACT

Two experiments were conducted to evaluate the nutritive values and maximum safe levels (MSL) of pennycress meal (PM) and canola meal (CM) for male broiler chicks housed in battery brooders. In experiment 1, a total of 480 chicks were fed either mash or crumbled diets containing 0, 5, 10, or 15% of PM for 18 days (8 diets; 6 replicates /diet). In experiment 2, a total of 660 chicks were fed diets containing 0, 3, 6, 9, 12 or 15% of either CM or PM for 14 days (11 diets; 6 replicates /diet). PM is a good source of protein (~ 31% CP) and it is very comparable to canola (~ 36% CP). However, it contains higher erucic acid (1.62 to 1.73%) and total glucosinolates (18.2 to 21.7 μmol /g). In experiment 1, increasing PM from 0 to 15% resulted in linear reductions ($P \leq 0.05$) in FI, BWG and FCR at 10 d. Linear and quadratic responses were observed for FI and BWG at 18 d, respectively. Crumbling feed resulted in improved BWG and FCR. No feed form by PM inclusion effects were observed. An estimated MSL of 10% PM based on orthogonal contrast analysis was optimal for satisfactory FI and BWG. The MSL was estimated by BLL and BLQ models to be 9.12 ± 0.50 and 7.0 ± 1.27, respectively. In experiment 2, linear reductions in FI, BWG and FCR due to PM inclusion were observed at 7 d. BWG at 18 d showed quadratic response to PM with no clear trends for FI or FCR. CM inclusion did not affect growth performance at 14 d suggesting the highest level to be safe. The MSL for maximum growth performance varied depending on the statistical analysis as follows: 12% by contrast and LSD, 15% by Scheffe's, 10.84 ± 0.57 by BLL and 8.61 ± 1.29 by BLQ. In conclusion, PM can be included in broiler starter diets as a protein source at a level of 7.0 ± 1.29 (based on BLQ model) without affecting the growth performance. Different statistical procedures could give different answers and this should be taken into account when analyzing the data.

Key words: Pennycress, Canola, Safe level, Broiler, Broken-line

INTRODUCTION

Field pennycress or simply pennycress (Thlaspi arvense L.) is a winter annual weed classified as a member of the Brassicaceae family commonly known as mustard family. The native habitat of pennycress is Eurasia but it can also be found in different regions of North America. Currently, pennycress is being evaluated as a potential biodiesel source due to the high oil content (~ 36% oil) in the seeds (Carr, 1993; Isbell, 2009; Fan et al., 2013). The oil is typically extracted from the seeds by expeller pressing (cold pressed) method which includes seed crushing, flaking and heat treatment prior to pressing (Selling et al., 2013). The collected oil is converted into methyl esters via transesterification process to be used as a biodiesel. The remaining portion after oil extraction is called press cake, oil cake or meal. Pennycress meal (**PM**) has been reported to be a good source of protein as it contains at least 26% CP (Selling et al., 2013; Hojilla-Evangelista et al., 2014). However, the meal also contains high levels of fiber, glucosinolates and erucic acid which can limit its nutritive value for poultry (Moser et al., 2009; Hojilla-Evangelista et al., 2014).

Canola is another oilseed crop belonging to the mustard family as pennycress. Unlike pennycress, canola contains low concentrations of glucosinolates and erucic acid after Canadian plant genetics developed new canola cultivars from rapeseed (Bell, 1982). Canola meal (**CM**) has been used as a protein source in poultry diets but its use is still limited due to the presence of antinutritional factors and high fiber content (Salmon et al., 1981; Slominski et al., 1999; Gopinger et al., 2014).

The maximum safe level (MSL) of CM in broiler diets can vary depending on several factors such as meal composition, processing method and the statistical analysis followed (e.g.

multiple range test, quadratic regression, etc.). Newkirk and Classen (2002) observed a quadratic response in feed intake and BW gain of broiler chicks at 19 d when feeding diets containing increasing concentrations of toasted or non-toasted canola meal (0, 6.3, 12.5, 20.8, 28.9 and 36.9 % of the diet). Woyengo et al. (2011) reported a linear decrease in feed intake and BW gain of male broiler chicks fed diets supplemented with either 0, 10, 20, or 40% expeller-extracted canola meal. Payvastegan et al. (2013) reported that feeding Solvent-extracted CM to broiler chicks up to 10% of the diet had no impact on 21-d growth performance and the reduction in performance was observed at 20% inclusion level as estimated by Duncan's multiple range test. The use of a multiple range test is very common in feeding trials due to probably the ease in performing the test. In fact, the use of the multiple range test is inappropriate in this type of feeding trials where the input factor is continuous (Petersen, 1977; Dawkins, 1983; Lowry; 1992; Pesti et al., 2009).

Recently, Alhotan et al. (2016) developed broken-line (spline) models to estimate the MSL of feed ingredients. This methodology can also be used in planning of feeding trials to increase experimental design efficiency by finding the optimal number of ingredient levels and replications. Evaluation of potential feedstuffs like PM or current cultivars like CM for poultry are desirable to increase efficiency of production. In addition, providing new methodologies for planning feeding trials and estimating the inclusion levels precisely are lacking. Therefore, the objectives of this study were to evaluate the nutritive value of PM and CM; to design a broiler feeding trial involving these ingredients based on broken- line methodology; and to estimate the MSL of PM and CM using various methods (multiple range tests, orthogonal contrasts and broken-line models) to illustrate how different conclusions can be drawn from the same data due to the choice of statistical analysis.

MATERIALS AND METHODS

General Procedures

Two experiments were conducted at the University of Georgia Poultry research facility (Athens, GA) using day-old Cobb 500 by-product male chicks obtained from Cobb Vantress Hatchery (Cleveland, GA). Chicks were reared in battery brooder units (Petersime Incubator Co.; Gettysburg, OH) and were provided with *ad libitum* access to feed and water. All experimental procedures followed were approved by the University of Georgia Institutional Animal Care and Use Committee. In experiment 1, dietary inclusion of PM and feed form (crumbles or mash) were investigated in an 18-day study. In experiment 2, the inclusion level of PM and CM were evaluated in a 14-day study after designing this trial based on data from experiment 1 and another experiment.

Experiment 1

Birds and Housing. A total of four hundred and eighty chicks were weighed and randomly assigned according to dietary treatment to 48 pens (8 dietary treatments; 6 replicates; 10 chicks in each pen). Chicks were fed four starter diets varying in PM inclusion level and provided in either crumbles or mash form. The room temperature was set at 30˚ C the first week and gradually decreased to 26˚ C thereafter. Brooders were the main source of heat supply and were switched off after one week of age. The photoperiod was 23 hours of light throughout the study. Birds and feed were weighed at 0, 10, and 18 day of age. Body Weight Gain (**BWG**), Feed Intake (**FI**), and Feed Conversion Ratio (**FCR**) were calculated.

Dietary Treatments. A corn- SBM basal diet was mixed with 0, 5, 10, or 15% of PM and prepared in two forms (crumbles or mash), totaling eight experimental diets (Table 7.1). Diets were formulated based on the true amino acid digestibility values for all ingredients which were obtained from a commercial AA database (Ajinomoto Heartland LLC, 2009) with the exception of PM. The digestibility coefficients for PM (Table 7.2) were obtained from a cecectomized rooster assay according to Parsons (1985). The PM used was supplied by the United States Department of Agriculture, USDA (Athens, GA) and referred to herein as PM1. All diets were formulated to meet or exceed the nutritional recommendations by Aviagen (Aviagen Inc., 2007).

Experiment 2

Study Planning. Planning of experiment 2 was done using the Maximum Ingredient level Optimization Workbook (MIOW) which is a simulation-based workbook. MIOW workbook requires some knowledge of the expected variation in the experiment and the shape of the "true" response to precisely estimate the MSL and the corresponding statistics of feed ingredients using broken-line models (Alhotan et al., 2015 and Alhotan et al., 2016). From previous experiments with PM (Experiment 1) and CM (data not shown), the coefficient of variation was about 5% and the true parameters of the response function were maximum gain = 590 g, MSL= 8, and rate constant = - 35 for PM and maximum gain= 625 g, MSL= 8, and rate constant = - 35 for CM. This information was used in MIOW workbook to find the efficient combination of levels and replications given the available resources at that time (i.e. 3 battery brooder units with 72 cages). This optimal combination should have a small SE of the MSL. Tables 7.3 and 7.4 show the possible combinations of levels and replications for PM and CM, respectively. The most effect design for both experiments was found to consist of 6 levels and 6 replications per level (36 pens in total) for PM and CM as the SE was reduced by about 38% compared to using 4 levels of 6 replications per level (36 pens in total). Therefore, this design was applied in experiment 2 using 6 levels (0, 3, 6, 9, 12 and 15%) and 6 replications per level.

Birds and Housing. In total, six hundred and sixty chicks were weighed and randomly assigned according to dietary treatment to 66 cages (11 dietary treatments; 6 replicate pens; 10 chicks in each cage). The initial room temperature was set at 33° C and was reduced gradually to 27° C at day 14. The room was equipped with forced air furnace as the only source of heat, a stirring fan and an exhaust fan. The photoperiod was maintained at 23 hours of light throughout the study. Birds and feed were weighed at days 0, 7, and 14 to calculate BWG, FI and FCR.

Dietary Treatments. Birds were fed mash diets containing 0, 3, 6, 9, 12 and 15% of either PM or CM. The meals were mechanically expeller-pressed and were supplied by Arvegenix Inc. (St. Louis, MO) for PM and Pacific Coast Canola (Warden, WA) for CM. The diets were corn- SBM basal diets containing corn, SBM, corn DDGS, poultry-by products and other ingredients (Table 7.5). The nutritional recommendations followed in feed formulation met or exceeded the nutritional recommendations established by Aviagen (Aviagen Inc., 2014). Feed formulation was based on standardized ileal AA digestibility coefficients from a commercial database (AminoDat 4.0, Degussa AG, Hanau-Wolfgang, Germany) for corn and SBM. The digestibility coefficients of DDGS and poultry-by products were obtain from NIR analysis.

Chemical Analysis

Representative samples were collected from PM1, PM2, CM and all diets used in both experiments and were finely ground to pass through a 1.18 mm sieve. PM1, PM2 and CM samples were analyzed for proximate composition (dry matter, crude protein (N x 6.25), crude fat, crude fiber and ash), Gross Energy (**GE**), Acid Detergent Fiber (**ADF**), Neutral Detergent Fiber (**NDF**) and amino acid (**AA**) concentrations at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). Dry matter (method 934.01, 2005), nitrogen (method 990.03, 2006), crude fat (AOAC method 920.39 (A), 1999), crude fiber (method 978.10, 2006) and ash (AOAC 942.05) were determined based on AOAC International (2000). NDF and ADF were determined according to Van Soest et al. (1991) and AOAC (method 973.18 (A-D), 2006), respectively. GE was determined using adiabatic bomb calorimeter. AA concentrations of the samples were analyzed based on AOAC (method 982.30 E (a, b, c), 2006). Fatty Acids in PM1, PM2, CM samples were determined by Gas Chromatography with Flame Ionization Detection (AOAC 939.05, AOCS 1e-91, AOCS Ce 2-66) by EPL Bio Analytical Services (Decatur, IL). Glucosinolate Contents in PM1, PM2, CM samples were determined using Ultra High Performance Liquid Chromatography with Ultra-Violet Detection (AOCS Ak 1-92, ISO 9167-1) by EPL Bio Analytical Services (Decatur, IL).

Statistical Analysis

Data from both experiments were analyzed using the GLM procedure of SAS software (SAS Institute, 2010). In experiment 1, the design of this study was a 4 x 2 factorial arrangement of treatments with 4 levels of PM1 (0, 5, 10, 15 %) and 2 levels of feed form (mash or crumbles). The two-way analysis of variance (**ANOVA**) was used to determine the effects of PM1 inclusion, feed form and any possible interactions. The design of experiment 2 was a completely randomized block design with 11 dietary treatments consisting of 5 inclusion levels of PM2 (3, 6, 9, 12, 15), 5 inclusion levels of CM (3, 6, 9, 12, 15) and a control diet with no PM2 or CM. Data were analyzed by one-way ANOVA. For both experiments, each battery brooder level was considered a block containing only one replicate cage as the experimental unit (10 chicks per cage). The effect of increasing PM1, PM2 and CM levels on the response variables were evaluated using polynomial contrasts. Mean separation was performed using orthogonal contrast, Fisher's LSD (Fisher, 1935), and Scheffe's test (Scheffe, 1953). The ITEM Workbook (Vedenov et al., 2015) was used to estimate the MSL by BLL and BLQ models. Differences were declared significant when $P \leq 0.05$.

RESULTS

Nutrient Composition

The total glucosinolate contents in PM1, PM2 and CM were 18.2, 21.7 and 3.12 µmol /g, respectively (Table 7.6). The predominant glucosinolate in PM1, PM2 was Sinigrin which reflects nearly all glucosinolate contents in the meals. The most abundant glucosinolates in CM were Gluconapin, Progoitrin and 4-hydroxyglucobrassicin which together compose more than 90% of the total glucosinolate contents in CM. The predominant fatty acids in pennycress meals are erucic acid, linoleic acid, oleic acid and alpha linolenic acid (Table 7.7). Whereas oleic acid and linoleic acid are the most abundant fatty acids in CM. Erucic acid content in CM was found to be very low (< 0.021). PM1 samples were found to have high fat (~ 9%) and high fiber (~ 17%). PM1 and PM2 samples contained about 31% CP and CM contained about 36% CP (Table 7.8). The AA patterns in CM, PM1 and PM2 are nearly identical (Table 7.8). The most abundant indispensable AA in the samples leucine while the least abundant is tryptophan. Among the dispensable, the most abundant AA is glutamic acid and cysteine is the least.

Experiment 1

Increasing PM1 inclusion level from 0 to 15% resulted in a linear decrease ($P < 0.05$) in FI for the first ten days of age, as well as for the entire period of study (Table 7.9). Similarly, BWG was reduced in a linear fashion for the first ten days of age. A quadratic response in BWG during 0-18 d was observed when increasing PM1 inclusion level from 0 to 15%. FCR was reduced linearly as a function of PM1 inclusion during 0-10 d with no trends observed during the entire period of study. The contrast statements revealed that PM1 inclusion at 5, 10 or 15% depressed FI during 0-10 d compared to the control and feeding 15% of PM1 resulted in a depression in cumulative FI. This was true for BWG when feeding all levels of PM1 reduced BWG during 0-10 d but only feeding the highest PM1 level reduced growth for 0-18 d as compared to the control. FCR was depressed only during 0-10 d at 10% or more of PM1 inclusion. Chicks fed the mash diets had reduced BWG compared to those fed the crumbled diets during both experimental periods studied. FCR was improved when the diets were crumbled compared to feeding the diets in mash form. There were no significant interaction effects between feed form and PM1 inclusion in this study. There were no differences ($P=0.533$) found in mortality rates due to PM feeding (data not shown). The mean mortality rate was about 2.5%. The MSL values for satisfactory BWG at 18 d as estimated by the ITEM workbook using treatment means were 9.12 ± 0.50 for BLL model and 7.0 ± 1.27 for BLQ model (Figure 7.1). The MSL values were estimated to be 8.23 ± 1.98 for BLL model and 4.22 ± 5.0 for BLQ model when all replicate pen means where used.

Experiment 2

When CM inclusion level was increased from 0 to 15%, a quadratic response in FI during the first week was observed (Table 7.10). BWG during this period responded similarly as weight gain decreased at an increasing rate and then increased at an increasing rate. No other regression trends were observed at any period examined. Contrast statements comparing each level to the controlled suggested that feeding 9% CM depressed FI and BWG during the first week of age. Results of feeding PM2 showed that increasing PM2 from 0 to 15% reduced FI linearly for the first week but not for the entire period as there was no clear trends (Table 7.11). BWG was reduced linearly by PM2 feeding at day 7 and 14. However, the response in BWG at day 14 could also be considered as quadratic instead of linear as the p-value was approaching significance (P=0.079). PM2 increases depressed FCR in a linear fashion at both 7 and 14 days of age. None of the levels of PM2 fed decreased FI based on the contrasts. Feeding 9% or above of PM2 depressed BWG and FCR at day 7. At day 14, BWG and FCR were depressed only at the highest level of PM2.

The MSL values for satisfactory BWG at 14 d based on the ITEM workbook using treatment means were 10.84 ± 0.57 for BLL model and 8.61 ± 1.29 for BLQ model (Figure 7.2). When all replicate pen means where used the estimates were 10.84 ± 1.93 for BLL model and 8.61 ± 2.70 for BLQ model. The MSL for BWG at 14 d based on Fisher's LSD test was found to be between 12 to 15% PM2 (Figure 7.3). Using Scheffe's test (Figure 7.4), the MSL was found to be 15%.

DISCUSSION

Nutrient Composition

The major antinutritional substances present in pennycress meal that could be limiting performance of broilers are glucosinolates and erucic acid ($22{:}1\Delta^{13}$). Pennycress meal samples used in this study contained high levels of glucosinolates as Sinigrin (18.2 and 21.7 µmol / g for PM1 and PM2, respectively) and erucic acid (1.73 & 1.62 % for PM1 and PM2, respectively). Hojilla-Evangelista et al. (2014) reported defatted pennycress meal to contain 36.71 ± 0.41 mg/g sample or 92.4 µmol / g of Sinigrin (Molar mass = 397.46 g/mol) which is almost 5 times greater than Sinigrin content in this study. In the current study, erucic acid was found to be the major fatty acid in pennycress followed by linoleic acid and this is in agreement with the findings of Moser et al. (2009) who reported pennycress oil to have high erucic acid (32.8 %) and linoleic acid (22.4 %). Canola meal, on the other hand, contained low levels of total glucosinolates (3.12 µmol / g) and erucic acid (< 0.021 %) which characterize the new cultivars of canola selected for low glucosinolate and erucic acid (Khajali and Slominski, 2012). The CP and AA pattern in pennycress samples are very comparable to canola. AA digestibility coefficients for PM2 are more comparable to CM than PM1. The digestibility coefficients for PM1 were generally lower than the digestibility coefficients of PM2. The differences between the coefficients of PM1 and PM2 could not be explained as there are many factors that can cause such variations (e.g. seed processing conditions, procedures followed in the rooster assays by different laboratories, analytical errors, etc.).

Experiment 1

FI was linearly reduced due to pennycress feeding and the highest inclusion of pennycress resulted in about 7% reduction in FI during the first ten days and 4% reduction in the cumulative FI. This reduction in FI was reflected in BWG as the weight gain decreased linearly during 0-10 d, but responded in a quadratic fashion reaching a maximum of 595 g at 5% PM then declining after that. The highest inclusion also resulted in about 10-point increase (depression) in FCR during 0-10 d only. In this experiment, synthetic AAs were added to the diets to meet the requirements of most limiting AAs, TSAA, lysine and threonine. Two other essential AAs became limiting as PM1 inclusion increased from 0 to 15%. Isoleucine and valine were reduced from 0.87 and 0.96 in the control diet to 0.80 and 0.90 in the highest inclusion diet, respectively. The synthetic forms of isoleucine and valine were not available to decrease this gap in requirements so, they were not added. In addition, the diets were maintained isocaloric and isonitrogenous and no minimum restrictions were set for these branched chain AAs. The reduction in growth performance seen at the highest PM1 inclusion could be partially due to either isoleucine or valine became the next limiting AA. Furthermore, the high fiber content in PM1 (17% CF) and the presence of antinutritional compounds such as glucosinolates and erucic acid may be other contributing factors to the reduction in performance. Feeding seed meals of the mustard family (e.g. camelina and canola), which can contain high concentrations of these compounds, have been reported to reduce feed intake and depress growth in poultry (Sim et al., 1985; Tripathi and Mishra, 2007). The glucosinolates *per se* are not toxic by themselves, but the toxic effects come from their metabolites (e.g. goitrin, nitriles, and thiocyanates) which are produced during processing or by microbial degradation in the gut under the actions of myrosinase (Khajali and Slominski, 2012). Ryhanen et al. (2007) reported a linear reduction in growth performance of 14-day broiler chicks fed graded levels of camelina sativa (0 to 10%)

containing 22.9 µmol / g of glucosinolates. Woyengo et al. (2011) observed similar reductions in growth performance of broiler chicks fed increasing levels of canola meal (0 to 40%) with 8.03 µmol / g of total glucosinolates.

Crumpling feed in this study did not improve FI but it did improve BWG and consequently FCR. The improvement in BWG of chicks fed the crumbled diets may be due to increasing net energy for gain as a results of reducing eating time (Abdollahi et al., 2013). In addition, the crumbling process includes heating the feed which may improve nutrient digestibility and detoxifying certain antinutritional compounds. Therefore, it might be possible that heating feed reduced the toxic effects of some toxic compounds in PM leading to improved growth. It has been shown that heating canola meal at 100˚ C inactivated the enzyme myrosinase improving the growth performance of broilers (Shires et al., 1983). Comparing each inclusion level to the control suggests that chicks become less sensitive to pennycress inclusion as they get older. Feeding 5% or above reduced FI and BWG during the first ten days of age. However, feeding this level produced similar performance as feeding no PM and the reduction in FI and BWG were observed at the highest level. Using the contrast method, the maximum safe level of PM for satisfactory growth is estimated to be 10%. Using the broken-line methodology, the maximum safe level is estimated to be 9.55 ± 0.04 for BLL model and 7.99 ± 0.12 for BLQ model.

Experiment 2

The main objectives of this experiment were to design a feeding trial where increasing levels of an ingredient is fed and to estimate the MSL of the ingredient using different methods (multiple range test, original contrasts and broken-line methodology) as explained by Alhotan et al. (2016). Planning feeding trials based on historical data (i.e. expected variation and response shape) should make the planning process to be based on science rather than tradition and guesswork. The reason of estimating the MSL using different methodologies was to show how conclusions drawn from such experiments can be influenced by different statistical methods. Feeding PM2 seems to agree with feeding PM1 in experiment 1 by causing a linear reduction in FI and BWG during the first period and a tendency of quadratic effect on the overall BWG. The cumulative FI does not agree with the data from experiment 1 as there were no linear reduction in FI in this experiment. However, the reduction is clearly observed above 9%. In general, the reduction in growth performance could be due to the high fiber content or the presence of glucosinolates and erucic acid as explained earlier. The glucosinolates content in PM2 in slightly higher than PM1 (21.7 vs. 18.2 µmol / g) while erucic acid is slightly lower (1.73 vs. 1.62%).

The only observation on feeding CM was the quadratic response in FI an BWG during the first week of age. Feeding CM seems to reduce FI and BWG gradually till 9% inclusion then increases in FI and BWG were observed at higher inclusions. The reduction in performance during this period especially at 9% could not be explained. Feeding up to 15% CM did not affect growth performance at either day 7 or 14. The analyzed contents of glucosinolates (3.12 µmol / g) and erucic acid (< 0.021 %) were very low and this could be one reason for the absences of any detrimental effects on performance.

The MSL of pennycress meal (experiment 2) varied depending on the statistical procedure used. Using a less conservative test like Fisher’s LSD to separate the means resulted in more significant differences with a MSL value between 12 to 15% for satisfactory BWG at 14 d. In contrast, using Scheffe's test (Figure 7.2), the MSL was found to be 15%. Scheffe's test is a very conservative test and resulted in no significant differences in this analysis. Multiple-range tests are very common in separating means in feeding trials of ingredients with increasing levels. The factor levels in this case is a continuous (quantitative) variable. Multiple-range tests are appropriate for discrete or qualitative factors, so they should not be used here. In addition, the multiple-range tests distinguish between two levels of the factors and do not give exact estimates or confidence intervals for the estimates. The broken-line methodology treats the input factor as continues and provide estimates for the confidence intervals (Alhotan et al., 2016). The broken-line methodology provides estimates of the MSL plus it is descriptive statistics. When treatment means were used to estimate the MSL by the broken-line models (observation to observation variation is not accounted for), the resulting MSL values were characterized with small SE and high R^2. When the variation was included by using the replicate pen means, the SE of the MSL increased and R^2 decreased. Introducing variation resulted in poorer fit and made it more difficult to fit the models to the data. This observation suggests minimizing the variation when designing feeding trials (e.g. more reps, blocking, etc.) to get accurate estimate of the MSL.

In conclusion, pennycress meal can be used as a source of protein in broiler diets and the MSL should be estimated accurately to minimize the impact of antinutritional factors (e.g. erucic acid and glucosinolates) on performance. The choice of the statistical analysis can influence the MSL estimates. Therefore, the right statistical analysis should be used to avoid any detrimental effects on performance.

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Table 7.1. Ingredients and calculated composition of the diets (Experiment 1)

	Pennycress Meal Inclusion Level, %			
	0	5	10	15
Ingredient, %				
Corn	50.51	46.99	43.46	39.93
Soybean Meal -48%	31.92	29.16	26.39	23.62
Pennycress Meal	0.00	5.00	10.00	15.00
Wheat	5.00	5.00	5.00	5.00
Corn DDGS	3.50	3.50	3.50	3.50
Poultry by-product	3.00	3.00	3.00	3.00
DL-Methionine	0.28	0.30	0.32	0.34
L-Lysine HCl	0.21	0.26	0.31	0.36
L-Threonine	0.07	0.08	0.10	0.12
Poultry Fat	2.74	3.96	5.17	6.39
Limestone	0.67	0.71	0.76	0.81
Defluor. Phos.	1.36	1.30	1.25	1.19
Common Salt	0.40	0.40	0.40	0.40
Vitamin Premix[1]	0.25	0.25	0.25	0.25
Mineral Premix[2]	0.09	0.09	0.09	0.06
Calculated composition				
ME, kcal/kg	3,025	3,025	3,025	3,025
CP, %	23.55	23.55	23.55	23.55
Ca, %	1.05	1.05	1.05	1.05
Nonphytate P, %	0.50	0.50	0.50	0.50
Digestable Lys, %	1.27	1.27	1.27	1.27
Digestable TSAA, %	0.94	0.94	0.94	0.94
Digestable Thr, %	0.83	0.83	0.83	0.83

[1]Vitamin premix provided the following (per kg of diet): thiamine mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; d-Ca pantothenate, 12 mg; vitamin B12,12.0 µg; pyridoxine-HCl, 2.7 mg; d-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-retinyl acetate, 5,500 IU; all-ractocopherol acetate, 11 IU; ethoxyquin, 150 mg.
[2]Trace mineral premix provides the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5; Se 0.20.

Table 7.2. True metabolizable energy and true amino acid digestibility coefficients (%) for canola meal and pennycress meal samples

Amino Acid	CM [1]	PM1[2]	PM2[3]
TMEn, kcal per Kg [4]	ND [5]	2,273	2,455
Amino Acid (%)			
Alanine	85.10	74.73	86.35
Arginine	91.70	81.23	89.74
Aspartic Acid	82.20	76.01	88.39
Cysteine	80.10	54.33	75.75
Glutamic Acid	91.60	79.08	88.6
Glycine	86.20	42.07	ND
Histidine	84.70	78.14	80.35
Isoleucine	85.50	80.05	85.29
Leucine	87.20	79.86	88.78
Lysine	70.70	69.68	77.54
Methionine	87.40	80.15	86.23
Phenylalanine	94.30	79.68	88.3
Proline	109.30	64.65	78.8
Serine	85.20	66.28	80.29
Threonine	79.50	69.13	82.18
Tryptophan	ND	ND	98.91
Tyrosine	98.20	78.62	87.64
Valine	83.80	69.7	85.39

[1] Mechanically expeller-pressed canola meal supplied by Pacific Coast Canola (Warden, WA)
[2] Mechanically expeller-pressed pennycress meal supplied by the United States Department of Agriculture, USDA (Athens, GA)
[3] Mechanically expeller-pressed pennycress meal supplied by Arvegenix Inc. (St. Louis, MO)
[4] True metabolizable energy kcal per kg in dry matter basis
[5]Not determined

Table 7.3. Simulations of different combinations of levels and replications to estimate the maximum safe level of pennycress meal by broken-line quadratic model (simulations= 100; CV= 5%)

Number of Pens	Reps	Levels	Min level	Max level	MSL[1]	SD	SE	95% CL		R^2
								Lower	Upper	
24	6	4	0	15	8.00	0.038	0.196	7.99	8.01	0.997
24	4	6	0	15	8.00	0.144	0.144	7.97	8.02	0.997
30	6	5	0	15	8.00	0.082	0.132	7.98	8.02	0.997
30	5	6	0	15	8.00	0.134	0.131	7.97	8.03	0.997
36	6	6	0	15	8.00	0.132	0.121	7.97	8.03	0.997
40	8	5	0	15	8.00	0.071	0.118	7.99	8.01	0.997
45	9	5	0	15	8.00	0.076	0.112	7.99	8.02	0.997
48	8	6	0	15	8.01	0.089	0.109	7.99	8.03	0.997

[1]Maximum safe level as estimated by the Maximum Ingredient level Optimization Workbook (MIOW).

Table 7.4 Simulations of different combinations of levels and replications to estimate the maximum safe level of Canola meal by broken-line quadratic model (simulations= 100; CV= 5%)

Number of Pens	Reps	Levels	Min level	Max level	MSL[1]	SD	SE	95% CL		R^2
								Lower	Upper	
24	6	4	0	15	7.99	0.036	0.200	7.99	8.00	0.997
24	4	6	0	15	8.00	0.146	0.142	7.97	8.03	0.997
30	6	5	0	15	7.99	0.083	0.138	7.97	8.00	0.997
30	5	6	0	15	8.01	0.132	0.128	7.99	8.04	0.997
36	6	6	0	15	8.00	0.109	0.122	7.98	8.02	0.997
40	8	5	0	15	8.00	0.079	0.120	7.98	8.02	0.997
45	9	5	0	15	7.99	0.070	0.114	7.98	8.01	0.997
48	8	6	0	15	8.00	0.114	0.110	7.98	8.03	0.997

[1]Maximum safe level as estimated by the Maximum Ingredient level Optimization Workbook (MIOW).

Table 7.5. Ingredients and calculated composition of the diets (Experiment 2)

		Canola Meal					Pennycress Meal				
Ingredient	0	3	6	9	12	15	3	6	9	12	15
Corn	55.99	55.19	54.38	53.58	52.78	51.98	54.17	52.36	50.55	48.73	46.92
Soybean Meal (48% CP)	32.74	30.42	28.10	25.79	23.47	21.16	31.01	29.28	27.55	25.82	24.09
Corn DDGS	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Poultry By-Product Meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Pennycress Meal	0.00	0.00	0.00	0.00	0.00	0.00	3.00	6.00	9.00	12.00	15.00
Canola Meal	0.00	3.00	6.00	9.00	12.00	15.00	0.00	0.00	0.00	0.00	0.00
Animal Fat	1.23	1.31	1.38	1.46	1.54	1.61	1.85	2.46	3.08	3.70	4.31
Dical. Phos.	1.13	1.11	1.10	1.09	1.07	1.06	1.10	1.08	1.05	1.03	1.00
Limestone	1.02	1.00	0.97	0.94	0.92	0.89	0.96	0.89	0.82	0.75	0.69
DL-Methionine	0.30	0.31	0.31	0.31	0.32	0.32	0.30	0.30	0.30	0.30	0.30
L-Lysine HCL	0.26	0.32	0.38	0.44	0.50	0.55	0.28	0.30	0.32	0.34	0.37
L-Threonine	0.10	0.11	0.13	0.15	0.17	0.19	0.09	0.09	0.09	0.09	0.09
Common Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin Premix [1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix [2]	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Calculated Composition											
M.E., kcal/kg	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Crude Protein, %	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Ca, %	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Avail. P, %	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
dLYS, %	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
dMET, %	0.63	0.63	0.64	0.64	0.64	0.64	0.63	0.62	0.62	0.62	0.61
dTSAA, %	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
dTHR, %	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.86

[1]Vitamin premix provided the following (per kg of diet): thiamine mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; d-Ca pantothenate, 12 mg; vitamin B12,12.0 μg; pyridoxine-HCl, 2.7 mg; d-biotin, 0.11 mg; folic acid, 0.55 mg; menadione sodium bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; trans-retinyl acetate, 5,500 IU; all-ractocopherol acetate, 11 IU; ethoxyquin, 150 mg.
[2]Trace mineral premix provides the following in milligrams per kilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5; Se 0.20.

Table 7.6. Glucosinolates contents in samples of canola and pennycress meal

Glucosinolates (µmol / g)[1]	CM [2]	PM1[3]	PM2 [4]
Sinigrin	<0.163	18.200	21.700
4-hydroxyglucobrassicin	0.671	<0.049	<0.049
4-methoxyglucobrassicin	<0.077	<0.077	<0.077
Epi-progoitrin	<0.071	<0.071	<0.071
Glucoalyssin	<0.068	<0.068	<0.068
Glucobrassicanapin	<0.390	<0.390	<0.390
Glucobrassicin	0.231	<0.059	<0.059
Glucoiberin	<0.176	<0.176	<0.176
Gluconapin	1.220	<0.090	<0.090
Gluconapoleiferin	<0.030	<0.030	<0.030
Gluconasturtiin	<0.124	<0.124	<0.124
Glucoraphanin	<0.050	<0.050	<0.050
Neoglucobrassicin	0.032	<0.020	<0.020
Progoitrin	0.967	<0.075	<0.075
Total Glucosinolates	3.12	18.20	21.70

[1]Micromoles per gram of sample

[2] Mechanically expeller-pressed canola meal supplied by Pacific Coast Canola (Warden, WA)

[3] Mechanically expeller-pressed pennycress meal supplied by the United States Department of Agriculture, USDA (Athens, GA)

[4] Mechanically expeller-pressed pennycress meal supplied by Arvegenix Inc. (St. Louis, MO)

Table 7.7. Fatty acids composition of samples of canola and pennycress meals

Fatty Acid (%)	CM [1]	PM1 [2]	PM2 [3]
C8:0 Caprylic Acid	< 0.020	< 0.020	< 0.020
C10:0 Capric Acid	< 0.020	< 0.020	< 0.020
C12:0 Lauric Acid	< 0.005	< 0.005	< 0.005
C14:0 Myristic Acid	0.007	< 0.005	0.006
C14:1 Myristoleic Acid	< 0.020	< 0.020	< 0.020
C15:0 Pentadecanoic Acid	< 0.020	< 0.020	< 0.020
C15:1 Pentadecenoic Acid	< 0.020	< 0.020	< 0.020
C16:0 Palmitic Acid	0.528	0.296	0.271
C16:1 Palmitoleic Acid	0.048	0.018	0.017
C17:0 Heptadecanoic Acid	0.006	< 0.005	< 0.005
C17:1 Hepta decenoic Acid	0.013	< 0.005	< 0.005
C18:0 Stearic Acid	0.192	< 0.041	< 0.041
C18:1 Oleic Acid	5.610	0.862	0.784
C18:2 Linoleic Acid	2.010	1.670	1.540
C18:3 Gamma Liniolenic Acid	< 0.021	< 0.021	< 0.021
C18:3 Alpha Linolenic Acid	0.708	0.711	0.641
C18:4 Stearidonic Acid	< 0.005	< 0.005	< 0.005
C19:0 Nonadecanoic Acid	< 0.005	< 0.005	< 0.005
C19:1 Nondecenoic Acid	< 0.005	< 0.005	< 0.005
C20:0 Arachidic Acid	0.056	0.016	0.016
C20:1 Eicosenoic Acid	0.094	0.136	0.596
C20:2 Eicosadienoic Acid	0.006	0.136	0.119
C20:3 Eicosatrienoic Acid	< 0.021	< 0.021	< 0.021
C20:4 Arachidonic Acid	< 0.021	< 0.021	< 0.021
C20:5 Eicosapentaenoic Acid	< 0.005	0.0137	0.008
C21:0 Heneicosanoic Acid	< 0.005	< 0.005	< 0.005
C22:0 Behenic Acid	0.029	0.012	0.013
C22:1 Erucic Acid	< 0.021	1.730	1.620
C22:5 N3 Docosapentaenoic Acid	< 0.005	< 0.005	< 0.005
C22:5 N6 Docosapentaenoic Acid	< 0.005	0.007	0.009
C22:6 Docosahexaenoic Acid	< 0.005	< 0.005	< 0.005
C23:0 Tricosanoic Acid	< 0.005	< 0.005	< 0.005
C24:0 Lignoceric Acid	0.0221	<0.0104	<0.0104
C24:1 Nervonic Acid	0.016	0.290	0.236

[1] Mechanically expeller-pressed canola meal supplied by Pacific Coast Canola (Warden, WA)
[2] Mechanically expeller-pressed pennycress meal supplied by the United States Department of Agriculture, USDA (Athens, GA)
[3] Mechanically expeller-pressed pennycress meal supplied by Arvegenix Inc. (St. Louis, MO)

Table 7.8. Proximate compositions (as is) and total amino acid contents for canola meal and pennycress meal samples

	CM[1]	PM1[2]	PM2[3]
Gross Energy	NA[4]	4,704	NA
Crude Protein	36.54	31.44	31.48
Fat	NA	9.53	NA
Fiber	NA	17.33	NA
Ash	NA	6.97	NA
Dry Matter	NA	91.55	NA
Indispensable AA			
Arginine	1.04	1.72	1.56
Histidine	0.68	0.63	0.60
Isoleucine	0.89	1.05	0.95
Leucine	2.61	1.79	1.70
Lysine	0.87	1.28	1.15
Methionine	0.54	0.38	0.36
Phenylalanine	1.12	1.18	1.08
Threonine	0.97	1.15	1.11
Tryptophan	0.19	0.34	0.32
Valine	1.18	1.38	1.31
Dispensable AA			
Alanine	1.75	1.18	1.50
Aspartic acid	1.67	1.98	1.94
Cysteine	0.53	0.45	0.61
Glutamic acid	4.18	3.68	3.84
Glycine	1.05	1.60	1.52
Proline	2.03	1.39	1.38
Serine	1.21	0.93	1.00

[1] Mechanically expeller-pressed canola meal supplied by Pacific Coast Canola (Warden, WA)
[2] Mechanically expeller-pressed pennycress meal supplied by the United States Department of Agriculture, USDA (Athens, GA)
[3] Mechanically expeller-pressed pennycress meal supplied by Arvegenix Inc. (St. Louis, MO)
[4] Not available

Table 7.9. Growth performance of broilers fed increasing levels of Pennycress meal (PM) in two forms (mash and crumbles) for 18 days (Experiment 1).[1]

Feed form	%	n	Feed Intake (g) 0 – 10 d		Feed Intake (g) 0 – 18 d		Body Weight Gain (g) 0 – 10 d		Body Weight Gain (g) 0 – 18 d		Feed Conversion Ratio (g/g) 0 – 10 d		Feed Conversion Ratio (g/g) 0 – 18 d	
Mash	0	6	257.6	4.4	749.5	10.2	223.3	3.5	589.3	9.2	1.15	0.00	1.27	0.02
	5	6	245.4	7.8	747.2	7.1	212.9	4.7	589.4	6.3	1.16	0.04	1.27	0.01
	10	6	238.4	4.8	734.8	26.4	197.9	2.9	574.0	13.5	1.20	0.02	1.28	0.03
	15	6	246.1	4.9	721.1	7.4	193.9	3.3	551.7	7.4	1.27	0.02	1.31	0.01
Crumbles	0	6	264.0	4.6	753.3	9.8	236.6	4.3	606.1	3.3	1.12	0.00	1.24	0.02
	5	6	253.9	1.5	748.4	4.7	225.6	1.9	597.0	4.4	1.13	0.01	1.25	0.00
	10	6	253.6	5.2	727.1	21.0	218.7	7.6	598.3	12.6	1.16	0.02	1.22	0.04
	15	6	240.2	5.5	719.5	12.3	200.1	6.1	568.3	10.6	1.20	0.02	1.27	0.01
<u>Main Effect Means</u>														
Mash		24	246.9	3.0	738.2	7.4	207.0	3.0	576.1	5.5	1.20	0.02	1.28	0.01
Crumbles		24	253.0	2.7	737.1	6.9	220.3	3.7	592.5	5.0	1.15	0.01	1.25	0.01
	0	12	260.9	3.2	751.4	6.8	224.6	5.5	591.8	7.3	1.14	0.01	1.26	0.01
	5	12	249.7	4.0	747.9	4.1	220.2	3.5	594.6	4.0	1.14	0.02	1.26	0.00
	10	12	246.0	4.1	731.0	16.1	212.0	4.7	588.8	9.2	1.18	0.01	1.25	0.03
	15	12	243.2	3.6	720.3	6.7	197.8	3.1	561.8	6.3	1.24	0.02	1.29	0.01
<u>Contrast</u>			------------------------------ Probabilities ------------------------------											
0 vs. 5			0.034		0.804		0.026		0.622		0.791		0.879	
0 vs. 10			0.006		0.158		<.0001		0.214		0.018		0.652	
0 vs. 15			0.001		0.034		<.0001		<.0001		<.0001		0.161	
ANOVA														
<u>Source</u>		df												
Form		1	0.099		0.914		0.007		0.017		0.002		0.015	
PM		1	0.001		0.016		<.001		0.002		<.0001		0.252	
PM^2		1	0.247		0.719		0.186		0.028		0.088		0.229	
Form x PM		1	0.352		0.778		0.379		0.279		0.401		0.527	
Error		42												

[1]Results are expressed as mean ± SEM.

Table 7.10. Growth performance of broilers fed increasing levels of canola meal for 14 days (Experiment 2).[1]

	%	N	Feed Intake (g) 0 – 7 d		Feed Intake (g) 0 – 14 d		Body Weight Gain (g) 0 – 7 d		Body Weight Gain (g) 0 – 14 d		Feed Conversion Ratio (g/g) 0 – 7 d		Feed Conversion Ratio (g/g) 0 – 14 d	
Canola Meal	0	6	136.2	2.5	502.6	11.8	119.1	2.9	382.9	14.9	1.14	0.02	1.32	0.04
	3	6	131.7	2.2	509.0	12.7	112.6	3.1	366.6	22.5	1.17	0.02	1.42	0.10
	6	6	131.1	3.5	504.3	15.5	112.9	5.7	387.5	16.1	1.17	0.03	1.31	0.04
	9	6	124.5	3.2	488.6	13.8	106.3	4.0	368.1	7.5	1.18	0.04	1.33	0.04
	12	6	134.0	3.4	502.1	12.3	116.5	1.7	387.8	7.2	1.15	0.03	1.30	0.01
	15	5	136.6	3.1	504.0	12.1	118.6	3.3	388.0	13.1	1.15	0.01	1.30	0.02
			----------------------------- Probabilities -----------------------------											
Linear			0.953		0.765		0.961		0.591		0.930		0.339	
Quadratic			0.012		0.685		0.022		0.562		0.311		0.718	
Cubic			0.748		0.491		0.955		0.823		0.574		0.347	
Contrast														
0 vs 3			0.289		0.728		0.214		0.431		0.408		0.191	
0 vs 6			0.231		0.926		0.234		0.820		0.462		0.877	
0 vs 9			0.009		0.452		0.018		0.474		0.359		0.877	
0 vs 12			0.592		0.981		0.611		0.812		0.890		0.757	
0 vs 15			0.927		0.940		0.916		0.811		0.819		0.817	

[1]Results are expressed as mean ± SEM.

Table 7.11. Growth performance of broilers fed increasing levels of Pennycress meal for 14 days (Experiment 2).[1]

		N	Feed Intake (g)				Body Weight Gain (g)				Feed Conversion Ratio (g/g)			
			0 – 7 d		0 – 14 d		0 – 7 d		0 – 14 d		0 – 7 d		0 – 14 d	
Pennycress	0	6	136.2	2.5	502.6	11.8	119.1	2.9	382.9	14.9	1.14	0.02	1.32	0.04
	3	6	134.0	4.1	508.2	16.7	116.4	5.0	391.4	12.6	1.16	0.02	1.30	0.03
	6	6	131.9	1.4	492.1	5.4	113.2	3.1	377.8	9.7	1.17	0.04	1.31	0.03
	9	6	130.6	2.3	516.5	9.3	108.9	2.4	383.2	4.0	1.20	0.01	1.35	0.02
	12	6	129.9	2.1	496.6	4.2	108.3	3.2	366.4	8.6	1.20	0.02	1.36	0.03
	15	5	129.0	3.7	471.6	16.1	105.0	2.8	334.5	23.5	1.23	0.03	1.43	0.09
			----------------------------- Probabilities -----------------------------											
Linear			0.036		0.128		0.001		0.009		0.003		0.044	
Quadratic			0.621		0.132		0.802		0.079		0.869		0.241	
Cubic			0.961		0.272		0.936		0.606		0.946		0.990	
Contrast														
0 vs 3			0.576		0.723		0.570		0.638		0.714		0.762	
0 vs 6			0.275		0.512		0.217		0.780		0.404		0.869	
0 vs 9			0.155		0.384		0.039		0.984		0.091		0.621	
0 vs 12			0.115		0.707		0.029		0.366		0.067		0.528	
0 vs 15			0.087		0.072		0.008		0.016		0.014		0.081	

[1]Results are expressed as mean ± SEM.

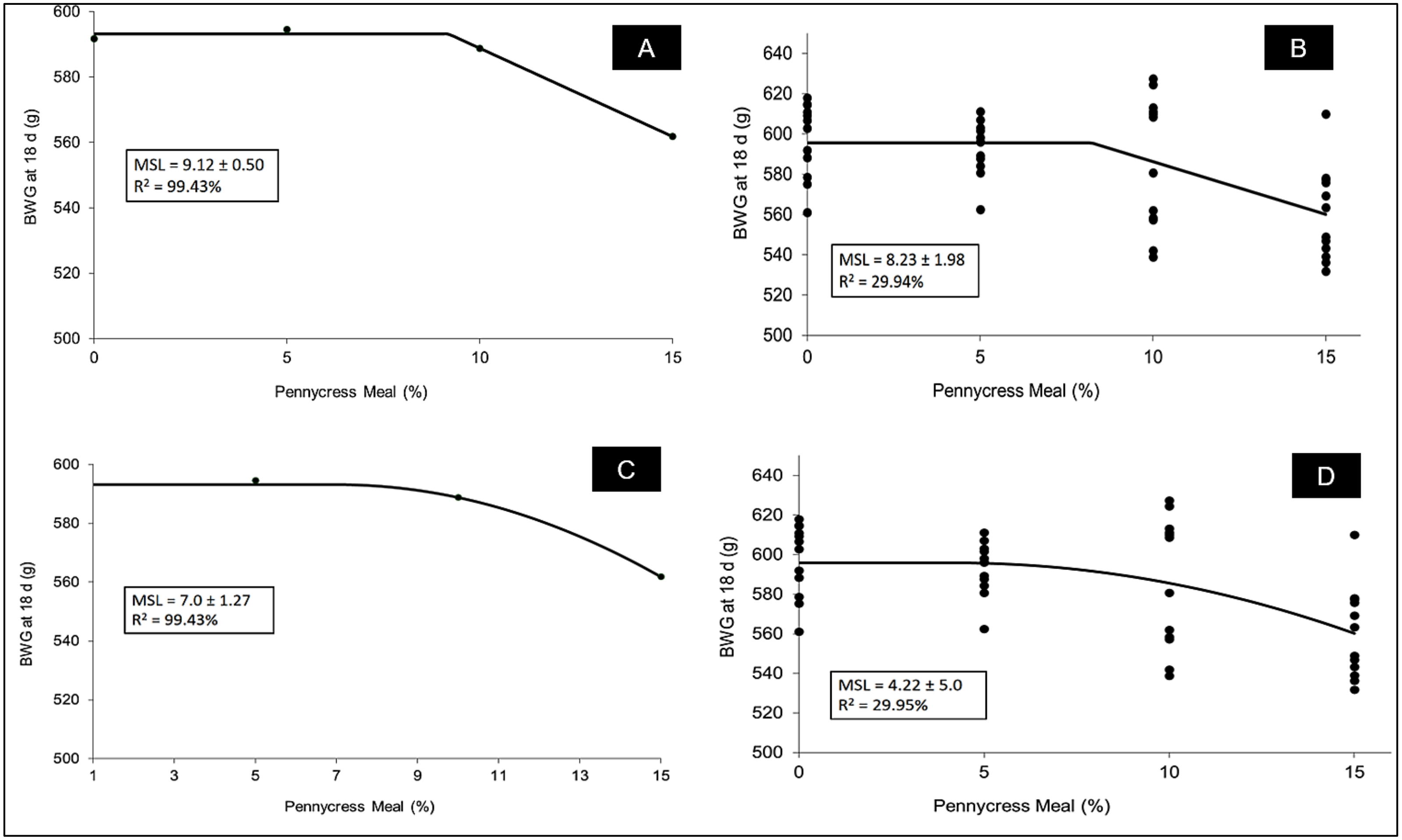

Figure 7.1. Estimating the MSL (mean ± SE) of pennycress meal for experiment 1 with the ITEM Workbook by two broken-line models with linear (BLL) and quadratic (BLQ) descending segments. MSL was estimated by BLL model using either treatment means (A) or all replicate pen means (B). MSL was estimated by BLQ model using either treatment means (C) or all replicate pen means (D).

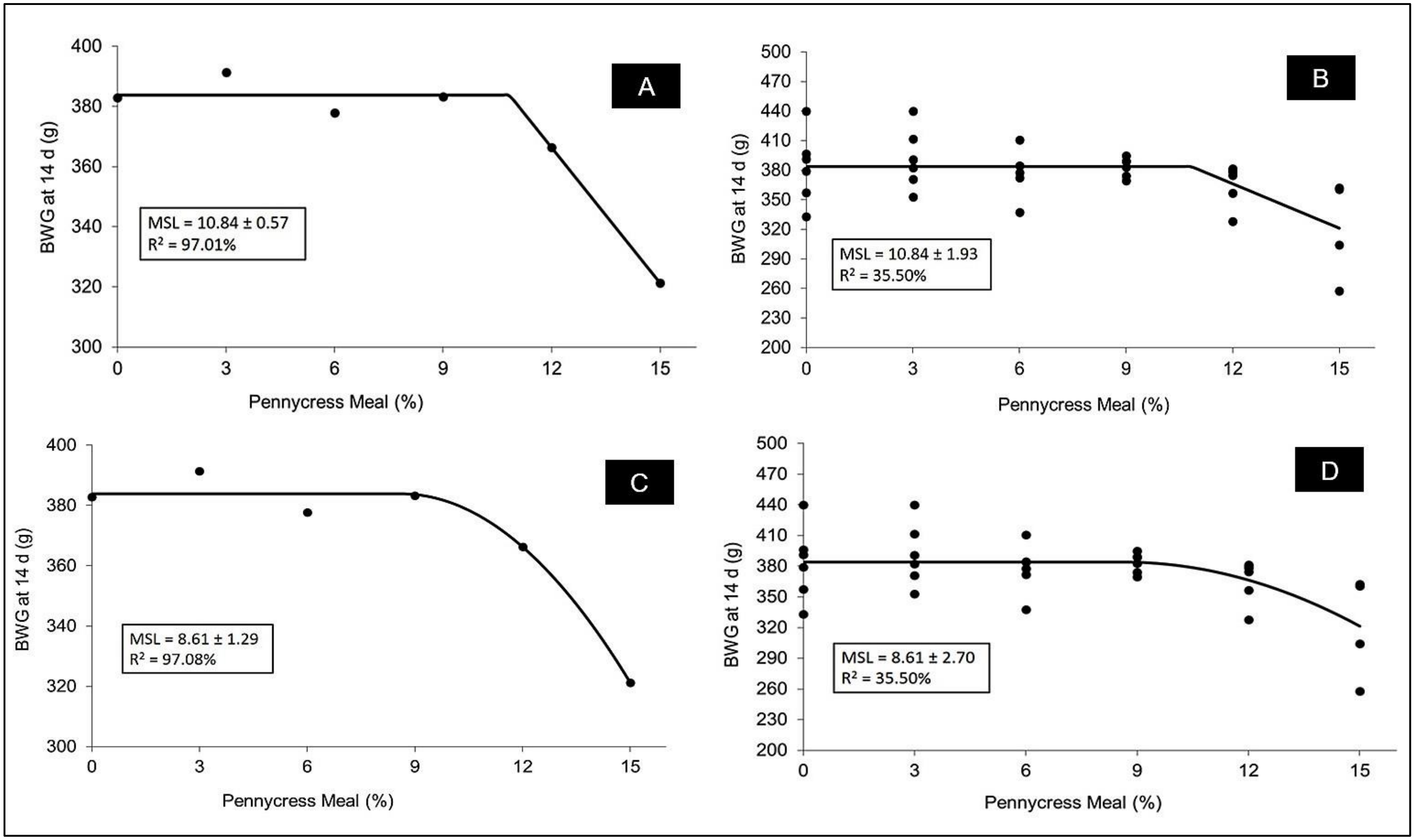

Figure 7.2. Estimating the MSL (mean ± SE) of pennycress meal for experiment 2 with the ITEM Workbook by two broken-line models with linear (BLL) and quadratic (BLQ) descending segments. MSL was estimated by BLL model using either treatment means (A) or all replicate pen means (B). MSL was estimated by BLQ model using either treatment means (C) or all replicate pen means (D).

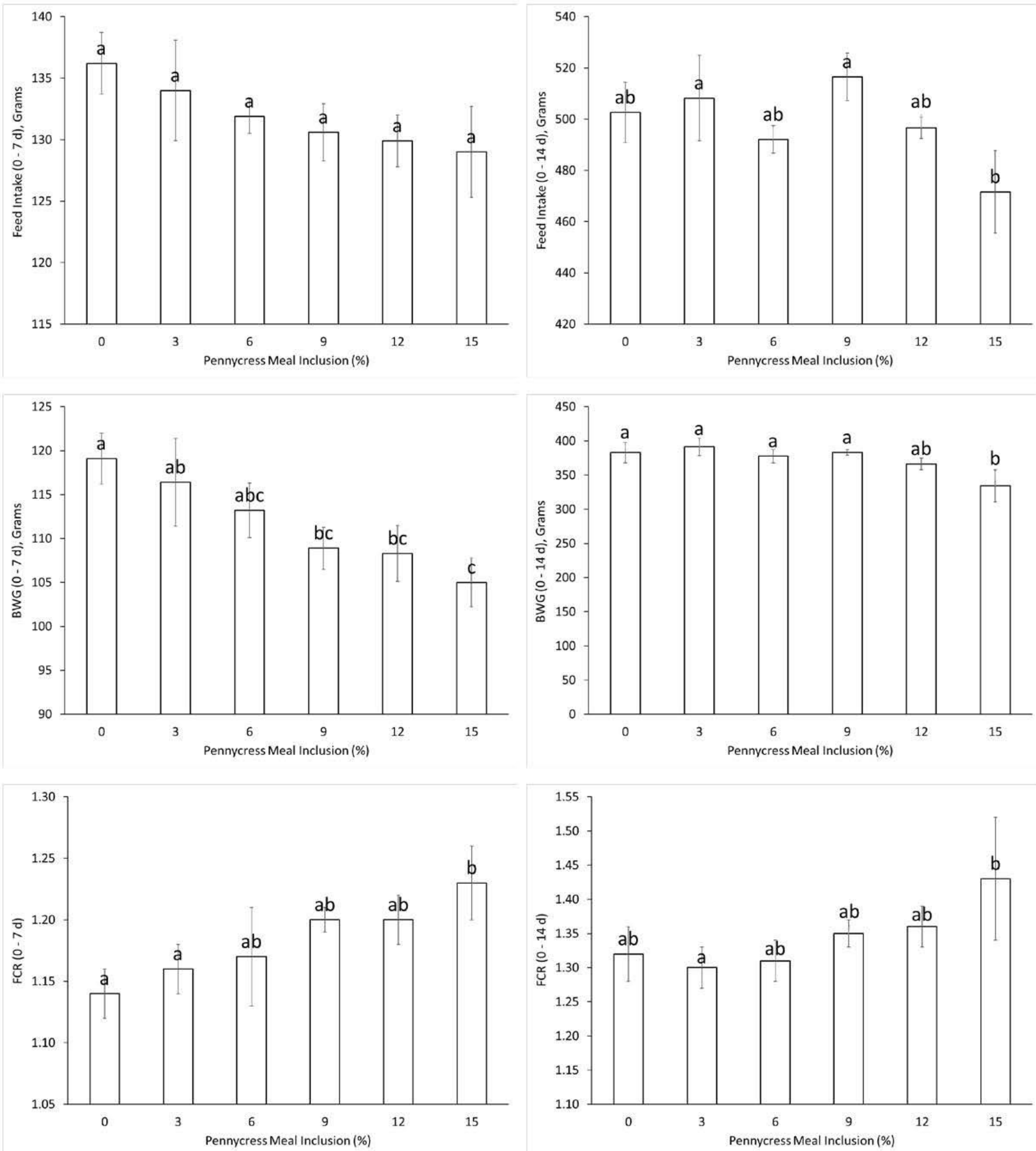

Figure 7.3. Growth performance data of Pennycress meal (PM2) feeding as analyzed by ANOVA and Fisher's LSD for mean separation. Means with the same letter are not significantly different.

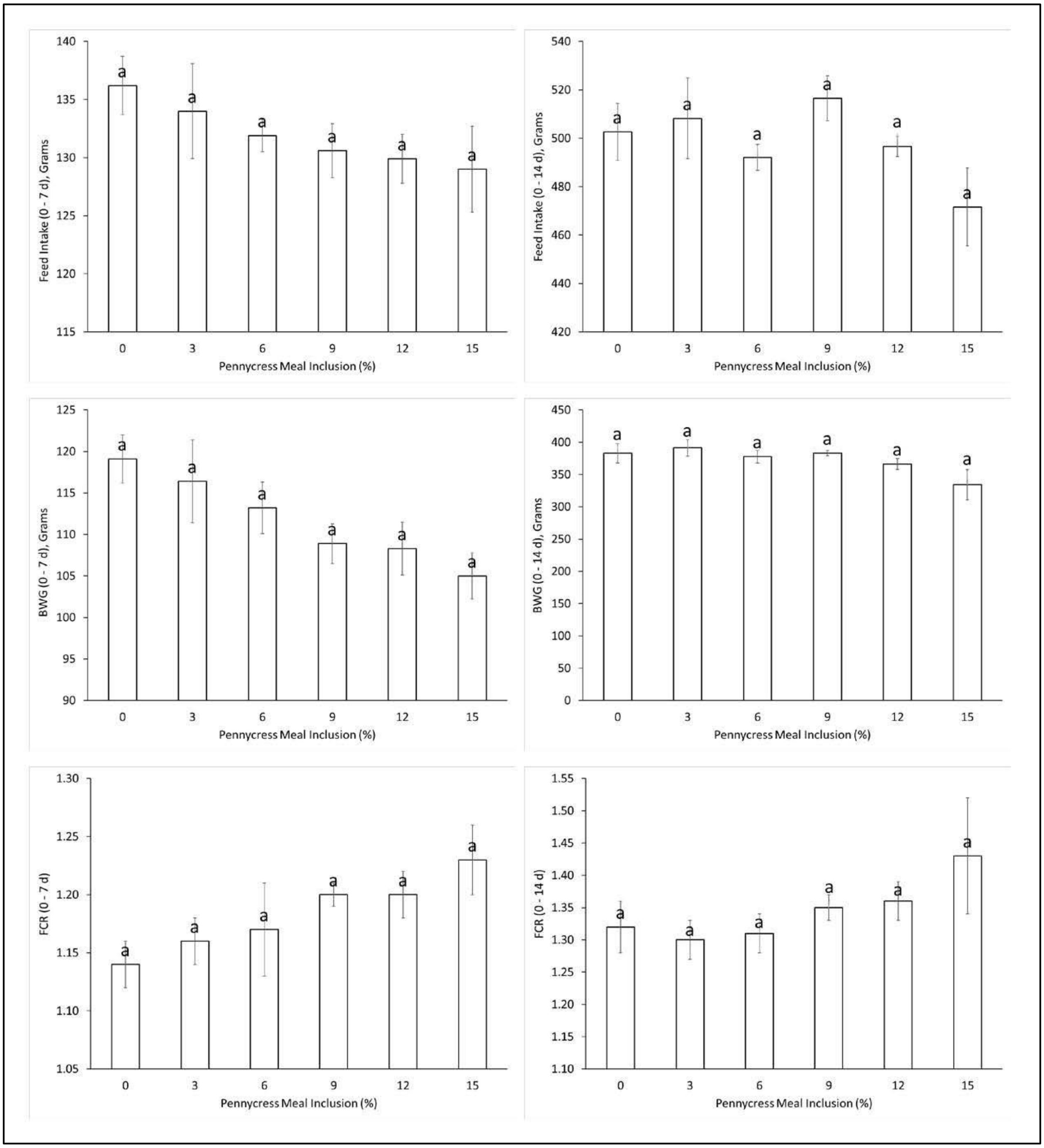

Figure 7.4. Growth performance data of Pennycress meal (PM2) feeding as analyzed by ANOVA and Scheffe's Test for mean separation. Means with the same letter are not significantly different

CHAPTER 8

GENERAL CONCLUSIONS

The ultimate goal of a more efficient poultry production system is to produce a cost-effective product in optimal conditions. However, there are multiple sources of efficiency that can limit the efficiency of such production system. Luckily, many sources of inefficiency can be overcome at the level of feed formulation. This dissertation evaluated several strategies that can be applied during feed formulation for maximum poultry performance and profitability. The results suggest that the feed formulation process can be improved by applying these strategies.

Despite the feed formulation model used (linear or non-linear), batch segregation strategy into below or above average bins was very effective in reducing nutrient variability at least 50%, compared to the traditional grain handling method of no segregation. Non-linear feed formulation increased costs of finished feed to guarantee the minimum specification of a nutrient at any confidence level. Employing the batch segregation strategy when using non-linear formulation models resulted in reducing feed costs.

Studying the relationship between dLys requirements and protein (true and crude) for maximum weight gain and FCR in broiler feeds demonstrated that the dlys requirements increased linearly as a function of the dietary protein contents. The dlys & true protein relationship resulted in a higher R^2 than the relationship of dlys & crude protein suggesting the true protein to be a better predictor of dlys requirement. For maximum weight gain, the dLys requirement using the broken-line linear model was estimated to be 4.92% ± 0.51 of the dietary true protein level. For, minimum FCR, the dLys requirement was estimated to be 5.58% ± 0.70. Using the dlys & true protein relationship to account for the requirements of all amino acids, especially in low protein diets, can be a good strategy to prevent any reduction in performance.

The choice of which digestible amino acid databases (chick assay vs. rooster assay) to use for maximum profitability was the main topic in chapter 5. The results suggest that feed formulation based on chick assay values are more profitable at low to moderate feed costs. When feed is cheap, meat value should be increased to maximize profitability when using the chick assay data. When feed is expansive, feed formulation based on the rooster assay seems to be more profitable at low meat values and large sizes. The only difference in performance found between the two groups was that FCR was improved when feeding diets formulated based on the chick assay.

Chapters 6 & 7 evaluated the effectiveness of broken-line linear (BLL) and broken-line quadratic (BLQ) in estimating the MSL in lieu of the traditional method the multiple range test. The results showed that BLL and BLQ provided good estimates of the MSL (small SE and high R2). The models also provided useful information to design future feeding trials. The most efficient design should have small SE of the MSL.

APPENDIX A: ESTIMATING CRUDE PROTEIN VARIABILITY AND SAVINGS OF BROILER FEEDS IN MICROSOFT EXCEL[1]

[1] Alhotan, R. A., G. J. Colson, and G. M. Pesti. 2014.The Georgia Agricultural Experiment Station, Athens.

SUMMARY

One source of inefficiency in poultry production comes from variation in feed ingredients. Using standard feed mixing techniques, grains and meals are each stored in their own bins (1-bin method). Linear programs are typically used to find the combination of ingredients meeting nutrient restrictions based on average ingredient compositions. To increase the probability of meeting nutrient restrictions greater than 50% of the time, stochastic models may be implemented. For instance, feed cost may be increased by 20% to meet the minimum crude protein requirement in 80% of batches instead of 50%. In-line equipment (such as NIR) is now available to quickly estimate ingredient compositions (e.g. % protein) and facilitate improved formulation techniques.

This paper describes Microsoft Excel workbooks designed to calculate 1) the effects of dividing ingredients into above- and below-average portions (2-bin method) and 2) the costs of providing nutrients at specified confidence levels. By dividing ingredients into above and below average portions, efficiency is increased by 1) improving performance due to under feeding with below specification batches of feed; and 2) minimizing waste from reduced over-feeding.

GENERAL OVERVIEW

Feedstuffs are characterized with inherent nutrient variability. When formulating poultry feeds using the standard linear feed formulation techniques, negative outcomes can be expected due to the inherent variability. This publication explains how to calculate and reduce measures of nutrient variability in feed formulated by linear techniques. Furthermore, calculation of the costs of providing nutrients at specified confidence levels by the non-linear (stochastic) techniques is discussed. Crude protein content of a corn-SBM broiler starter diet has been chosen herein as an example. Microsoft Excel workbooks have been constructed to achieve the objectives of this publication.

How is Poultry Feed Formulated?

The majority of poultry feeds are formulated by least-cost feed formulation software which is based on linear programing. Linear programming software formulates feeds with only 50% assurance of meeting nutrient requirements (half the batches will be below average). Stochastic programming can also be used to formulate feeds with 99.99% or even higher assurance of meeting the requirement of any nutrient. The Stochastic programming method takes into account nutrient variability. In practice, feeds currently formulated by either linear or stochastic programming methods are formulated from feed ingredients each stored in its own, single bin.

What is the Importance of Estimating Nutrient Variability in Poultry Feeds?

Batches of feed ingredients arrive at feed mills from different sources. The batches are often not analyzed for the actual content of nutrients and feeds are formulated based on the expected nutrient averages, ignoring the inherent nutrient variability in feedstuffs. As a result, feeds formulated based on historical averages may only meet the nutrient requirements of the birds 50% of the time with high variation in meeting the requirements. Estimating nutrient variability in finished batches of feed would be beneficial to feed formulators to overcome the problem of nutrient variability.

How is Crude Protein Variability Estimated?

A large number of ingredient samples collected from poultry producers in North America have been analyzed for their proximate composition including crude protein (CP) (Tahir et al., 2012). Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA) were constructed with thousands of simulated batches of feed to estimate CP variability of feeds using two grain handling methods. The two methods are (1) Feed formulation from undivided, unseparated batches of corn and soybean meal (SBM) (1-bin method) and (2) feed formulation from batches of corn and SBM separated into above and below averages (2-bin method). The 2-bin method is proposed to reduce nutrient variability in finished batches of feed, improve live performance, reduce input costs and decrease waste and environmental impact.

Why is the Simulation Method Used?

The distributions of CP in corn (mean = 6.9%; SD = 0.59) and SBM (mean = 47.51%; SD = 1.42) samples in Figures 1 and 2 do not seem to be normal when the data are graphed. Using an average value does not represent the true value of feed ingredient tables that may be delivered to a mill. Monte Carlo simulation was used to represent the CP data as normal distributions by matching the mean and standard deviation of the observed data with the simulated distribution. Figures 1 and 2 demonstrate the shapes of the distribution curves before and after Monte Carlo simulation.

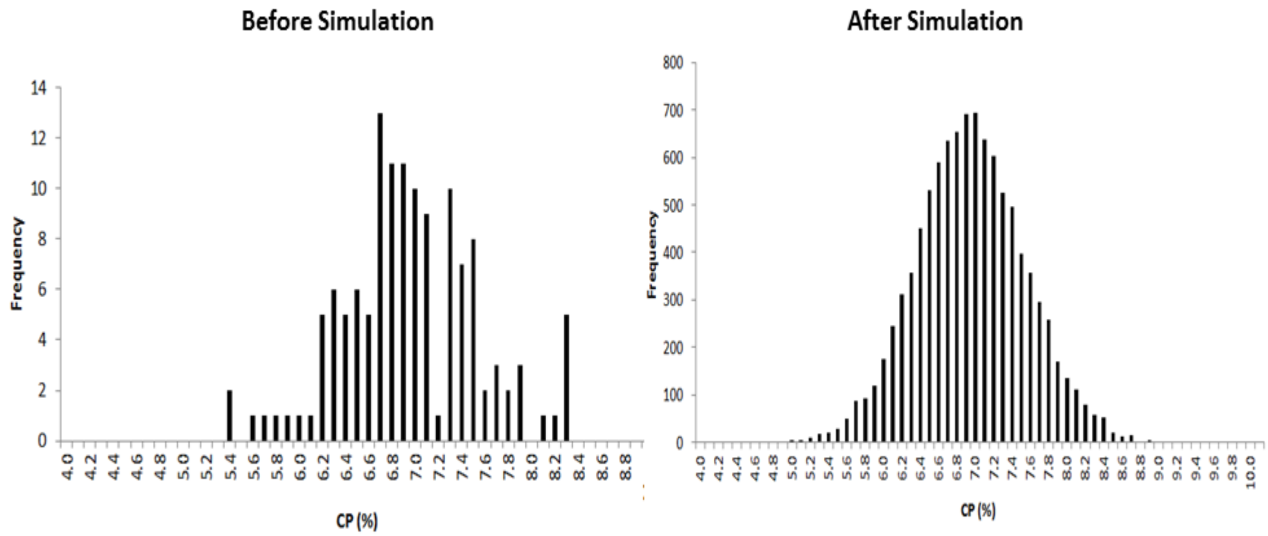

Figure 1. Corn crude protein (CP) distribution before and after Monte Carlo simulation

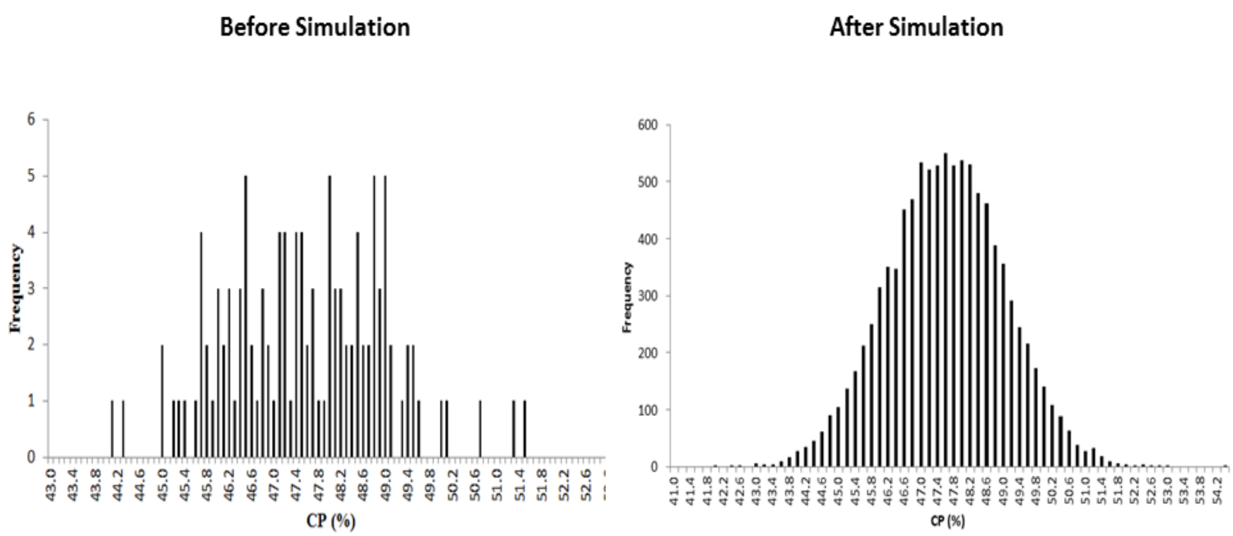

Figure 2. Soybean meal crude protein (CP) distribution before and after Monte Carlo simulation

How to Estimate CP Variability for Feeds Formulated by a Linear Programming Model and the 1-Bin Method?

1. Spreadsheet Construction

The workbook, named "CP-VEW1", was constructed for this purpose (click here to download CP-VEW1 workbook; click here to download CP-VEW1 Tutorial). The worksheet titled "Simulations" contains thousands of simulated batches of finished feed (Figure 3). The numbered cells in column B (B9:B10008) represent the ID numbers of 10,000 batches of finished feed. The entries in cells C9 through C10008 and D9 through D10008 are simulated CP values for corn and SBM, respectively. These simulated values were generated using the Monte Carlo simulation method. The simulation process was done using the function NORMINV (RAND (), CP population mean of the ingredient, CP population standard deviation of the ingredient). The entries in cells E9 through M10008 are the quantities of feed ingredients that make up the feeds (i.e. corn, SBM, poultry fat, limestone, dicalcium phosphate, salt, vitamin premix, mineral premix and dl-methionine). Each row is a simulation of one batch of finished feed formulated with the WUFFDA workbook (WUFFDA, 2004), which is linear programming software, using the average CP of corn (6.9%) and SBM (47.51%). It should be noted that the quantity of each feed ingredient is the same among the batches of feed/rows to reflect the real life situation when feeds are formulated based on the average CP values of the ingredients. Column N (N9:N10008) represents the total amount of each batch of feed calculated by summing the amount of the ingredients in each row. Column O shows the formula cost in dollars for each batch of feed based on the ingredient prices listed in cells F3 through K3. The dietary CP value for each batch of feed is listed in column P; calculated from the three protein sources in the feeds (corn, SBM and Dl-methionine). Column Q shows the level of dietary CP in each batch of feed; if it is equal to or above 23%, then number 1 is assigned to this level and if it is below this level, the assigned number is 0. CP means and standard deviations for corn and SBM being investigated are entered in cells C4 through D5. The results of this worksheet appear in cells M4 through R6. The mean, standard deviation, and coefficient of variation of all the CP values in column P are displayed in cells N4 through N6. The proportion of the batches of feed that lie above any desired CP level in cell D6 is displayed in cell P6. The number and percentage of batches that meet the specified minimum are displayed cells R5 and R6. Data for CP are graphed in separate worksheets. Histograms were generated by using the histogram tool of the analysis toolpak. The worksheet titled "G1" shows the distribution of corn CP in column C while the worksheet "G2" shows the distribution of SBM CP in column D. CP levels in the 10,000 batches of finished feed are graphed in the worksheet titled "G3".

CP Values to be Simulated		
Statistics	Corn	SBM
Mean (%)	6.90	47.51
SD	0.59	1.42
CP Level Tested		23.50

Ingredient Prices $ per 100 lb					
Corn	16.00	DCP	20.00	DL.Met	220.00
SBM	28.00	Salt	2.78		
Poultry Fat	34.00	Vit. mix	370.00		
Limestone	3.00	Min. mix	57.00		

Feed Formulation Results					
Statistics	%			Feeds Mixed	10,000
Mean	23.00	CP Level Tested	23.50	# of Meeting 23%	4,997
SD	0.65	Z-Value	0.77	% of Meeting 23 %	49.97
CV	2.80	% Above Tested Level	21.97	Formula Cost	$23.53

ID	Corn CP (%)	SBM CP (%)	Corn (%)	SBM (%)	Poultry Fat (%)	Limestone (%)	DCP (%)	Salt (%)	Vit. mix (%)	Min. mix (%)	DL-Met (%)	Total (%)	Formula Cost ($)	Diet CP (%)	CP level
1	6.63	46.90	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.62	0
2	7.40	49.21	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.94	1
3	7.72	49.06	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	24.03	1
4	6.16	48.86	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.21	1
5	6.19	47.78	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.78	0
6	6.75	45.34	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.03	0
7	6.81	46.68	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.61	0
8	6.85	47.37	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.92	0
9	7.44	48.38	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.61	1
10	6.08	44.84	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.51	0
11	8.06	48.35	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.90	1
12	7.57	48.41	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.69	1
13	6.95	47.73	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.11	1
14	6.99	47.08	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.87	0
15	6.65	48.37	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.24	1
16	6.93	47.54	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.03	1
17	5.88	47.76	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.62	0
18	6.02	46.57	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.20	0
19	6.24	47.52	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.69	0
20	6.33	44.58	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.52	0
21	6.23	45.58	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.89	0
22	6.49	47.79	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.92	0
23	6.95	47.83	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.15	1
24	6.77	44.81	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.82	0
25	6.58	46.37	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.38	0
26	5.70	46.26	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	21.92	0
27	6.61	48.88	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	23.43	1
28	6.57	47.85	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.98	0
29	7.47	45.51	47.33	41.25	7.31	1.40	1.62	0.45	0.25	0.15	0.24	100.00	23.53	22.45	0

Title | Simulations | G1 | G2 | G3

Figure 3. The "simulations" worksheet of the CP-VEW1 workbook to estimate CP measures of variation of feeds formulated by the 1-bin method

2. Understanding the Results of the "CP-VEW1" Workbook.

Feeds formulated in this example were intended to meet the requirements of broiler starter feeds (NRC, 1994) at a CP level of 23%. All the feeds were formulated at corn CP of 6.9 % and SBM CP of 47.51%. It is possible to modify this workbook to be used for any stage of production of any species by reformulating the feed according to the nutritional requirements of the stage or species of interest and then the ingredients' quantities can be transferred to this workbook. One objective of this work was to estimate CP variability in the finished feed and to know the distribution of CP in the batches as well. In our example, the entries in cells C4 through D5 produced the results in cells M4 through R6. The mean CP of the 10,000 batches of finished feed is approximately 23% which is the specified CP level in this example. The measures of variability of CP for the finished feed are standard deviation (SD) ≈ 0.64 and coefficient of variation (CV) ≈ 2.79. The percentage of batches of feed that lie above any CP level can be determined by entering any value in cell D6 and the result will appear in cell P6. For instance, if we want to know the percentage of batches of feed that lie above 20%, we simply enter 20 in cell D6 and recalculate the worksheet (press the F9 key) to get the value of 100% in cell P6. The specified CP of 23% was achieved approximately 50% of the time, as shown in cell R5, which is what we expect when we formulate feeds with linear programing techniques (Figure 4; G3 worksheet). The average formula cost of the feeds is presented in cell R6. It should be noted that the outputs change slightly as the formulas in this spreadsheet are recalculated either automatically or manually. With 100 simulations the fluctuations are considerable, with 10,000 the fluctuations are quite small. The number of simulations can be adjusted to meet the needs of the operator subject to the speed of the operator's computer.

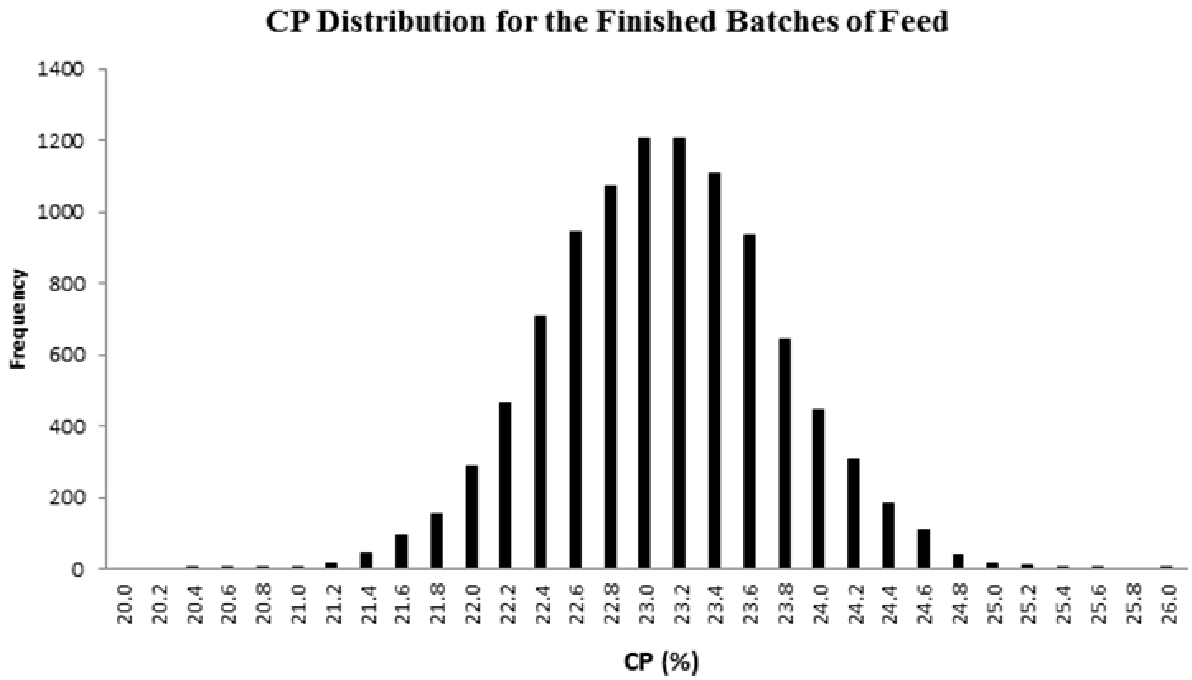

Figure 4. CP distribution for the finished batches of feed formulated by the 1-Bin Method

How to Estimate CP Variability for Feeds Formulated by a Linear Programming Model and the 2-Bin Method?

1- Spreadsheet Construction

Modern technology allows for the rapid estimation of the nutrient content of feed ingredients. It is possible to divert above and below average rail car loads to bins designated as the low CP portion (below-average) or high CP portion (above-average) and then formulate the feed. The feed was formulated with equal portions of each ingredient (i.e. 50% below-average and 50% above average for each of corn and SBM) and this step was done by forcing WUFFDA to use the new portions of the same ingredient in 1:1 ratios. It should be noted that a new CP mean (truncated distribution mean) was used in the ingredient matrix of WUFFDA with each portion. To determine these new means, the Excel spreadsheet named "TND Calculator" was constructed to generate 1,000 CP simulations for each of corn and SBM (click here to download TND Calculator). These simulated CP values were grouped based on the mean CP of each ingredient into "low CP" values (below the mean; assigned 0 in columns J and K) or high CP values (above the mean; assigned 1 in columns J and K) and the new means were obtained for each group as presented in cells L3 through M6. The workbook "CP-VEW2" is similar to "CP-VEW1" with the exception of grouping of the simulated CP values in columns K and L into "low CP" or "high CP" values (grouped into columns M through P) based on corn and SBM population means (Figure 5) (click here to download CP-VEW2 workbook; click here to download CP-VEW2 Tutorial). The number of the batches of finished feed in this workbook is fewer (n = 2,500) than CP-VEW1 workbook because it takes more time to conduct the simulations. Feed ingredient quantities obtained from WUFFDA are listed in columns Q through AA. All the ingredients that constituted the batches of feed in this workbook are totaled in column AB. Formula cost, dietary CP, and the level of CP for the batches of feed are presented in columns AC, AD, and AE, respectively. The ingredient characteristics (mean and SD) are entered in cells N4 through O5 and the results are in cells Z4 through AD6. The histograms of CP can be found in other worksheets.

CP Values to be Simulated		
Statistics	Corn	SBM
Mean (%)	6.90	47.51
SD	0.59	1.42
CP Level Tested	25.00	

Ingredient Prices $ per 100 lb					
Corn	16.00	DCP	20.00	DL.Met	220.00
SBM	28.00	Salt	2.78		
Poultry Fat	34.00	Vit.mix	370.00		
Limestone	3.00	Min.mix	57.00		

Statistics
Mean
SD
CV

	Corn CP (%)	SBM CP (%)	Low Corn CP (%)	High Corn CP (%)	Low SBM CP (%)	High SBM CP (%)
10	7.27	47.36	5.58	7.27	47.36	47.98
11	5.58	47.31	6.03	7.06	47.31	49.23
12	6.03	47.98	6.41	6.90	45.53	48.19
13	6.41	45.53	6.60	7.28	44.19	47.75
14	6.60	44.19	6.45	7.50	45.68	49.20
15	7.06	45.68	5.98	6.92	46.73	48.66
16	6.90	46.73	6.80	7.22	47.12	47.60
17	7.28	49.23	6.51	7.15	44.12	48.40
18	7.50	47.12	6.90	7.02	46.43	47.86
19	6.92	44.12	6.80	7.16	46.79	48.08
20	6.45	48.19	5.75	7.16	47.41	49.09
21	7.22	47.75	6.55	7.44	45.50	48.35
22	7.15	46.43	6.40	7.01	47.05	47.68
23	5.98	46.79	6.74	7.37	47.18	49.11
24	6.80	47.41	6.63	7.58	47.13	47.87
25	7.02	45.50	6.85	7.48	47.13	47.61
26	7.16	49.20	6.71	8.43	47.00	48.06
27	6.51	48.66	6.83	7.85	46.35	48.22
28	7.16	47.05	6.22	7.57	47.32	49.99
29	7.44	47.60	6.03	7.64	47.07	48.56
30	7.01	48.40	6.16	7.29	46.98	48.61
31	7.37	47.86	5.79	6.94	46.37	50.18
32	6.90	48.08	6.07	7.84	46.61	49.09
33	7.58	47.18	6.34	7.00	46.09	50.40
34	7.48	47.13	6.12	7.17	44.35	48.99
35	6.80	49.09	6.68	7.47	45.86	48.67
36	8.43	48.35	6.25	6.95	45.37	50.22

	High CP Corn (%)	Low CP Corn (%)	High CP SBM (%)	Low CP SBM (%)	Poultry Fat (%)	Limestone (%)	DCP (%)	Salt (%)	Vit. mix (%)
10	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
11	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
12	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
13	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
14	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
15	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
16	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
17	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
18	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
19	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
20	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
21	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
22	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
23	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
24	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
25	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
26	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
27	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
28	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
29	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
30	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
31	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
32	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
33	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
34	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
35	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25
36	23.67	23.67	20.62	20.62	7.31	1.40	1.62	0.45	0.25

Figure 5. The "simulations" worksheet of the CP-VEW2 workbook to estimate CP measures of variation of feeds formulated by the 2-bin method

2. Understanding the Results of the "CP-VEW2" Workbook

As in CP-VEW1, means and SDs for CP populations being studied are entered in the upper left-side of this worksheet (cells N4 through O5) and the results are displayed in the upper right-side (Z4 through AD6). The same entries for CP statistics were used in this workbook. The results show that the CP average for the batches of feed is again 23% (Z4) but the SD is much lower than with the 1-bin method ~ 0.27 (Z5). The percentage of batches of feed above any CP value of interest can be obtained in the same way as in the CP-VEW1 workbook. For example, if 22.5% is entered in cell N6, the percentage of batches above this level are ~ 96.30%. The percentage meeting the CP in the feed is roughly the same as in CP-VEW1 workbook (~ 50%; cell AD5). CP values of the batches of feed (column AD) when graphed are characterized with a tall and narrow distribution (Figure 6) compared to Figure 4.

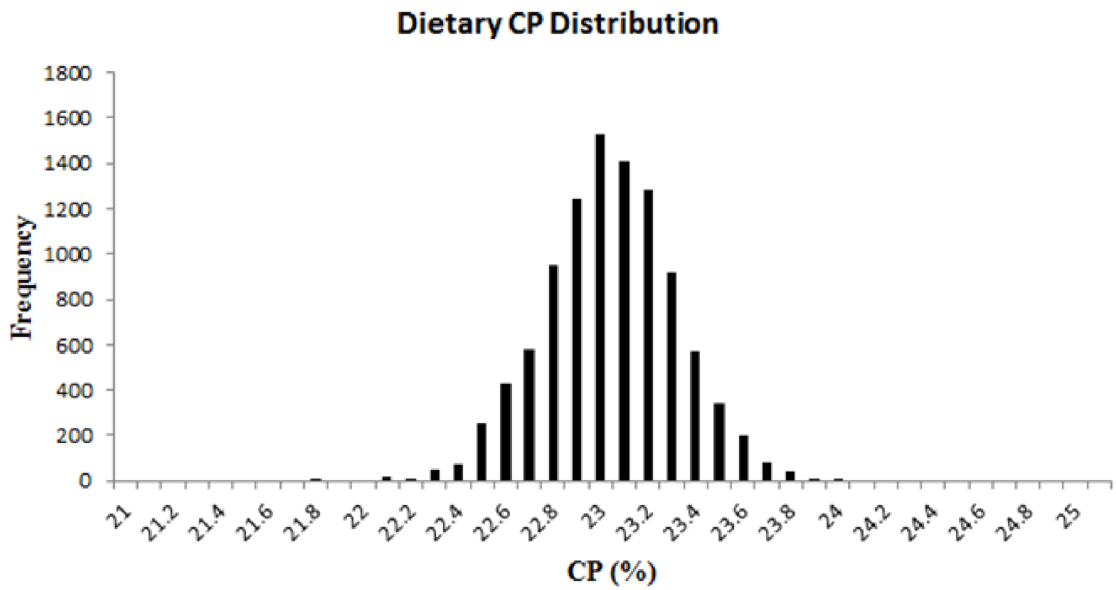

Figure 6. CP distribution of the finished batched of feed formulated by the 2-Bin Method

How to Determine the Level of CP in the Feed being formulated at any Probability Level?

1. Spreadsheet Construction

To determine directly and practically the level of CP at any given probability when formulating feed, two automated worksheets were constructed. The worksheets calculate the standard deviation and the CP content of the feed being formulated at any probability level. For ease, the worksheets were implemented into the WUFFDA workbook to obtain these important pieces of information during feed formulation. The workbook named CP Estimator 1 (Figure 7) is designed to be used with the 1-bin method while the workbook CP Estimator 2 is designed for the 2-bin method (click here to download CP Estimator1; click here to download CP Estimator2). In the worksheet "simulations" of the workbook CP Estimator 1, the entries in cells B4 through B8 are CP means and SDs for corn and SBM being used in WUFFDA. Any probability values between 0 and 1 can be entered in cell B9. The amounts of corn and SBM obtained from WUFFDA formulations are updated in cells B14 and B15. The expected CP in feed and its SD are presented in cells B19 and B20, respectively. The CP content related to the probability entered in cell B9 is presented in cell B22. Column D contains the IDs of the simulated CP values for corn (column P) and SBM (column Q). Column S represents the final CP content for each of the simulated feeds. The workbook CP Estimator 2 is very similar to CP Estimator 1 except the CP simulations were grouped into low- or high-CP populations as presented in columns R through U.

2. Understanding the Results of 'CP Estimator" Workbooks.

After the feed is formulated normally with WUFFDA, the amounts of corn and SBM used in the formula should appear in the corresponding cells of the simulations sheet. The DL-methionine column in the simulations worksheets is also updated. The next step is to input any probability value in cell B9 to determine the level of CP associated with this value. For example, if we decide to use 0.9 in cell B9 of the workbook CP Estimator 1, the result in cell B22 after worksheet recalculation (the F9 key for windows users) will be 22.17%. There is a 90% probability that the actual CP average in the feed being formulated meets or exceeds 22.17%. The CP value in cell B19 represents the specified CP limit in the feed, which is 23% in this example. The calculated standard deviation is ~ 0.65 for the 1-bin method and ~ 0.28% for the 2-bin method (cell B20). These statistics are very similar to those obtained by the workbooks CP-VEW1 and CP-VEW2.

POPULATION CHARACTERISTICS	CP (%)
Corn Population Mean	6.90
Corn Population Stdev.	0.59
SBM Population Mean	47.51
SBM Population Stdev.	1.42
Probability	0.50

OPTIMIZATION	
LBS of Corn from in Mixture	47.33
LBS of SBM from in Mixture	41.25

RESULTING MIXTURE	
Expected Protein Content	23.01
Standard Deviation	0.65
Protein Content at threshold probability	23.01

				Mixed Distribution
ID of Simulated Value	Corn Population Distribution	SBM Population Distribution	DL-Metionine in Finished Feed	Mixture Proten Content (Corn 1+Corn 2+SBM1+SBM2+ Met)
1	6.29	48.18	0.24	22.99
2	6.68	48.10	0.24	23.14
3	5.81	45.99	0.24	21.86
4	6.02	45.52	0.24	21.76
5	7.61	48.74	0.24	23.84
6	6.49	46.03	0.24	22.19
7	6.37	47.89	0.24	22.90
8	6.94	47.12	0.24	22.86
9	8.42	47.12	0.24	23.56
10	6.78	47.02	0.24	22.74
11	5.88	48.09	0.24	22.76
12	5.89	47.50	0.24	22.52
13	6.50	47.27	0.24	22.71
14	7.91	47.36	0.24	23.42
15	6.82	48.05	0.24	23.19
16	7.33	44.97	0.24	22.16
17	7.00	46.12	0.24	22.48
18	6.29	47.03	0.24	22.51
19	7.00	46.94	0.24	22.81
20	7.50	45.98	0.24	22.66
21	6.77	46.26	0.24	22.42
22	6.96	46.13	0.24	22.46
23	7.12	46.34	0.24	22.62
24	6.57	47.70	0.24	22.92
25	7.73	49.29	0.24	24.13
26	6.37	47.72	0.24	22.83
27	6.78	46.46	0.24	22.51
28	6.74	50.86	0.24	24.30

Title / Ingredients / Nutrients / Formulate / **Simulations** / Graphs / Feed Spec / Mixing Sheet / Ingredients (Poultry) / Ingredients (Feeds

Figure 7. Calculation of standard deviation and CP content of the feed at any probability given by CP Estimator 1 workbook

What Grain Handling Method Minimizes CP Variability, the 1-Bin Method or the 2-Bin Method?

As seen from the results of the workbooks (Table 1), formulating feeds with the 2-bin method resulted in a significant reduction in the standard deviation and CV of CP in finished feeds compared to formulating feeds with the regular 1-bin method. The standard deviation for feed formulated by the 2-bin method was approximately 0.27 (CV≈ 1.18) while the standard deviation for the 1-bin method was 0.64 (CV≈ 2.79). The distribution of the CP values around the mean was altered, as well. Almost 96 % of the batches of feed for the 2-bin method lie above 22.5% compared to only 78 % for the 1-bin method. On the other hand, only 3.5 % of the batches of feed for the 2-bin method lie above 23.5% compared to 22% for the 1-bin method. The majority of the batches of feed (≈ 93%) formulated with the 2-bin method have CP values within 1 percentage point (between 22.5 and 23.5). In contrast, only 56 % of the batches of feed for the 1-bin method fall within this percentage point. The reduction in the number of batches having very low CP contents (CP < 22.5%) can support the growth performance of all birds. On the other hand, the reduction in the number of batches having excessive CP content (CP > 23.5%) can reduce nitrogen pollution to the environment.

Table 1. Effect of grain handling method on crude protein variability and the percentage of batches of feed that lies above certain CP levels

	Grain Handling Method	
CP Statistics	**1-Bin Method**	**2-Bin Method**
Mean, %	23.00	23.00
Standard deviation, %	0.65	0.28
Coefficient of variation %	2.81	1.20
CP level (%)	………….. % above CP level…………	
21.0	99.90	100.00
21.5	98.98	100.00
22.0	93.89	99.98
22.5	78.00	96.34
23.0	49.96	49.20
23.5	21.94	3.35
24.0	6.09	0.01
24.5	1.01	0.00
25.0	0.10	0.00
25.5	0.01	0.00

How to Determine the Cost of Providing CP at Specified Confidence Levels for Feeds Formulated by Stochastic Programming and the 1-Bin Method?

1- Spreadsheet Construction

The stochastic programming spreadsheet in Figure 8 (SPW1) was constructed based on Pesti and Seila (1998) (click here to download SPW1 workbook; click here to download SPW1 Tutorial). The ingredients as well as their prices ($/ 100 lbs.) used above were used here (cells B1 to L1) and (cells B2 to L2). The weight of each ingredient appears in cells B3 to L3. CP values of the ingredients and the corresponding standard deviations are presented in cells B4 to L4 and B5 to L5, respectively. The entries in cells B6 through L19 are the nutrient compositions of the feed ingredients used based on NRC (1994). The quantity of each ingredient used in the formula is presented in cells B21 to L21. The outputs in cells B24 to L24 indicates the cost of each ingredient used. The minimums and maximums of the ingredients are specified in cells B22 to L22 and cells B23 to L23, respectively. The formula cost ($/100 lbs.) is presented in cell B25. Column M contains the nutrient specification and column N contains the maximum amount of each nutrient to be used. The supplied amount of each nutrient in the final formula is output in column O. A stochastic constraint was implemented into the spreadsheet and the constraint is $\sum_{j=1}^{n} \mu_{ij}\, x_j \,+ Z_i \sqrt{\sum_{j=1}^{n} \sigma_{ij}^2\, x_j^2} \;\geq b_i$ where u_{ij} is the mean of the ith nutrient in the jth ingredient; x_j is the fraction of the jth ingredient; Z_i is the standard normal deviate of the ith nutrient; σ_{ij}^2 is the variance of the ith nutrient in the jth ingredient; and b_i is the confidence level of meeting the ith nutrient (D'Alfonso et al 1992). The ith nutrient in this example is CP. The first part of the constraint ($\sum_{j=1}^{n} \mu_{ij}\, x_j$) is the total CP of the formula while the second part ($Z_i \sqrt{\sum_{j=1}^{n} \sigma_{ij}^2\, x_j^2}$) is the product of multiplying the Z_i value by the square root of the summed cells in row 20. The value of this constraint is computed by the formula in cell O4. The Z_i value is displayed in cell B26 and is calculated based on the probability value in cell B28, which is the desired probability of success in meeting the specified protein level. Column P calculates the average content of each nutrient. To optimize the stochastic formulation problem, the solver option must be selected. Once selected, a dialog box is produced which contains the objective value (formula cost) that needs to be minimized and subject to a set of constraints (Figure 9). The solving method selected in the dialog box is GRG Non-Linear since the problem to be solved is not linear (stochastic). The stochastic problem is optimized by clicking solve and the solver results dialog should appear.

	A	B	C	F	G	H	I	J	K	L	M	N	O	P
1		Corn	SBM	Poultry fat	Limestone	DCP	Vitamin premix	Mineral premix	salt	DL-Met	MIN (Nutrient)	MAX (Nutrient)	Supplied	Average content
2	Cost ($)	16.00	28.00	34.00	3.00	20.00	370.00	57.00	2.78	220.00				
3	weight	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4	CP	6.90	47.51	0.00	0.00	0.00	0.00	0.00	0.00	57.52	23.00	100	23.00	23.00
5	CP SD	0.59	1.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.87	0.87
6	ME	3.35	2.44	8.20	0.00	0.00	0.00	0.00	0.00	3.61	3.20	100	3.20	3.20
7	Ca	0.02	0.27	0.00	38.00	21.30	0.00	0.00	0.30	0.00	1.00	100	1.00	1.00
8	NPP	0.13	0.40	0.00	0.00	18.70	0.00	0.00	0.00	0.00	0.45	100	0.45	0.45
9	TSAA	0.29	1.28	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.90	100	0.90	0.90
10	Met	0.14	0.62	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.50	100	0.56	0.56
11	Cysteine	0.15	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.34	0.34
12	Lysine	0.20	2.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10	100	1.28	1.28
13	Arginine	0.32	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	100	1.55	1.55
14	Valine	0.32	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90	100	1.08	1.08
15	Tryptophan	0.06	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	100	0.29	0.29
16	phenylalanine	0.33	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	100	1.15	1.15
17	Threonine	0.24	1.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	0.87	0.87
18	isoleucine	0.23	2.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	0.99	0.99
19	Histidine	0.19	1.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	100	0.58	0.58
20	sigma^2*Xj^2	0.08	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
21	Quantities	0.48	0.41	0.07	0.02	0.01	0.00	0.00	0.00	0.00				
22	MIN (Ingredien	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
23	MAX(Ingredien	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00				
24	Cost/ Ingredie	7.63	11.53	2.44	0.05	0.24	0.93	0.09	0.01	0.53				
25	Formula cost $	23.45												
26	Z value	0.00												
27	Probability	0.50												

SPM | Stochastic | Outputs

Figure 8. Stochastic programming workbook “SPW1” based on the 1-bin method

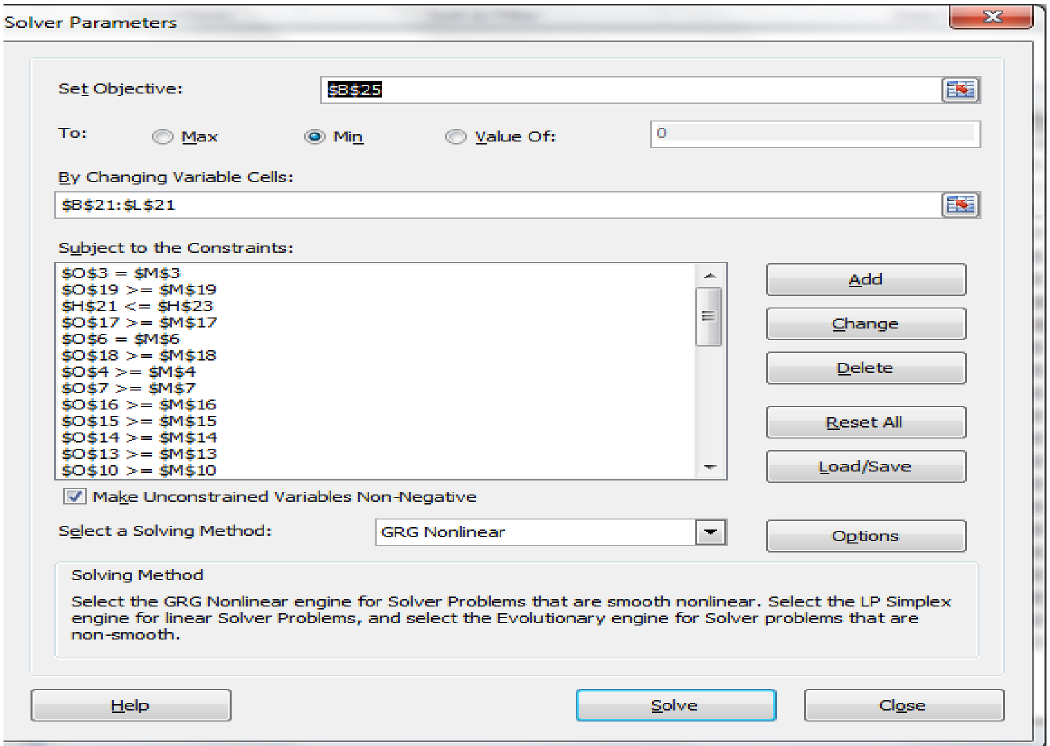

Figure 9. The solver parameters dialog box of the stochastic programming spreadsheet

2- Understanding the Results of SPW1

The workbook SPW1 is designed to calculate the average content of CP in feeds formulated by the 1-bin method at any confidence level and to estimate the cost of feed at that confidence level. For example, to be 60% confident that the feed contains at least 23% (in cell O4) we simply enter 0.6 (in cell B27) and optimize the formulation problem by clicking solve to get 23.17% (in cell P4) and the formula cost is $23.50 (in cell B25). In other words, to be 60% sure the feed contains at least 23% the average content has to be increased to 23.17%. When the confidence level is increased to 80%, the average CP content required increases to 23.56% leading to increased formula cost ($23.63).

How to Determine the Cost of Providing CP at Specified Confidence Levels for Feeds Formulated by Stochastic Programming and the 2-Bin Method?

1- Spreadsheet Construction

The workbook SPW2 (Figure 10) is very similar to SPW1 except that corn and SBM was divided into two equal portions as discussed previously (click here to download SPW2 workbook; click here to download SPW2 Tutorial). Corn was divided into low CP corn (Column B) and high CP corn (Column C) while SBM was divided into low CP SBM (Column D) and high CP SBM (Column E). Two more rows each were added for corn (rows 7 and 8) and SBM (rows 10 and 11) to force the program to use the two portions of each ingredient in a ratio of 1:1 (cells B7 and C8 for corn portions; cells D11 and E10 for SBM portions). The corresponding CP and SD for each portion discussed previously were used to formulate feeds by SPW2.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Low Corn	High Corn	Low SBM	High SBM	Poultry fat	Limestone	DCP	/itamin premi	Mineral premi:	salt	DL-Met	4IN (Nutrient	4AX (Nutrient	Supplied	ıverage conten
2	Cost ($)	16.00	16.00	28.00	28.00	34.00	3.00	20.00	370.00	57.00	2.78	220.00				
3	weight	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4	CP	6.40	7.39	46.41	48.66	0.00	0.00	0.00	0.00	0.00	0.00	57.52	23.00	100	23.00	23.24
5	CP SD	0.36	0.37	0.85	0.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00			0.53	0.53
6	Corn CP	-1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
7	Low Corn	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.24	0.00
8	High Corn	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.24	0.00
9	SBM CP	0.00	0.00	-1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00
10	High SBM	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.21	0.00
11	Low SBM	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.21	0.00
12	ME	3.35	3.35	2.44	2.44	8.20	0.00	0.00	0.00	0.00	0.00	3.61	3.20	100	3.20	3.20
13	Ca	0.02	0.02	0.27	0.27	0.00	38.00	21.30	0.00	0.00	0.30	0.00	1.00	100	1.00	1.00
14	NPP	0.13	0.13	0.40	0.40	0.00	0.00	18.70	0.00	0.00	0.00	0.00	0.45	100	0.45	0.45
15	TSAA	0.29	0.29	1.28	1.28	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.90	100	0.90	0.90
16	Met	0.14	0.14	0.62	0.62	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.50	100	0.56	0.56
17	Cysteine	0.15	0.15	0.65	0.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.34	0.34
18	Lysine	0.20	0.20	2.88	2.88	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10	100	1.30	1.30
19	Arginine	0.32	0.32	3.40	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.25	100	1.57	1.57
20	Valine	0.32	0.32	2.25	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90	100	1.09	1.09
21	Tryptophan	0.06	0.06	0.64	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	100	0.29	0.29
22	phenylalanine	0.33	0.33	2.40	2.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	100	1.16	1.16
23	Threonine	0.24	0.24	1.83	1.83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	0.88	0.88
24	isoleucine	0.23	0.23	2.14	2.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80	100	1.00	1.00
25	Histidine	0.19	0.19	1.19	1.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	100	0.59	0.59
26	sigma^2*Xj^2	0.01	0.01	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
27	Quantities	0.24	0.24	0.21	0.21	0.07	0.02	0.01	0.00	0.00	0.00	0.00				
28	MIN (Ingredier	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
29	MAX(Ingredier	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00				
30	Cost/ Ingredie	3.76	3.76	5.85	5.85	2.47	0.05	0.24	0.93	0.09	0.01	0.52				
31	Formula cost $	23.52														
32	Z value	-0.84														
33	Probability	0.80														

SPM | Stochastic | Ourputs

Figure 10. Stochastic programming workbook "SPW2" based on the 2-bin method

2- Understanding the Results of SPW2

The workbook SPW2 is designed to calculate the average content of CP in feeds formulated by the 2-bin method at any confidence level and to estimate the cost of feed at that confidence level. This workbook can be optimized in the same way as in SPW1 as discussed previously. To be 80% sure the finished feed has at least 23% the average CP content has to increase to 23.24% with a formula cost of $23.52. In the same manner, the cost of feed at any confidence level can be determined.

What Grain Handling Method Maximizes Savings, the 1-Bin Method or the 2-Bin Method?

Feed formulation using the 2-bin method costs more at low probabilities of success (P < 50%) and less at high probabilities of success (P > 50%) compared to feed formulation using the

1-bin method (Table 2). For example, at P= 1% formula cost increases $5.38 per ton but at P= 99% formula cost decreases $6.47 per ton with the 2-bin method compared to the 1-bin method. In practice, no one should formulate feed at low probability of success. The 2-bin method can be an economically efficient way to reduce formula costs. Normally when we buy something that is labeled to contain a certain amount of anything, we do not expect to receive less than that 50% of the time. Separating feed ingredients into different categories helps reduce the amount of sub-standard feed; and stochastic programming demonstrates the cost of achieving a minimum specification.

Table 2. Probability of success of meeting the specified crude protein level in broiler feeds, feed costs, and the expected savings when feeds are formulated by a stochastic programming

	1-Bin Method		**2-Bin Method**		
Probability of Success (%)	**Average CP (%)**	**Cost ($/ Ton)**	**Average CP (%)**	**Cost ($/ Ton)**	**Savings ($/ Ton)**
1	21.57	459.21	22.37	464.58	-5.38
5	21.97	461.94	22.55	465.81	-3.87
10	22.19	463.33	22.65	466.48	-3.04
20	22.46	465.28	22.77	467.29	-2.01
30	22.66	466.63	22.86	467.87	-1.24
40	22.84	467.80	22.93	468.38	-0.58
50	23.00	468.91	23.00	468.86	+0.05
60	23.17	470.03	23.07	469.33	+0.70
70	23.35	471.25	23.15	469.85	+1.40
80	23.56	472.70	23.24	470.45	+2.24
90	23.86	474.74	23.36	471.30	+3.44
95	24.12	476.47	23.46	472.00	+4.47
99	24.61	479.82	23.66	473.34	+6.47

What Can be Concluded from the Linear and Stochastic Workbooks?

Formulating poultry feeds using the 2-bin method based on linear programming will greatly decrease CP variability (the CV is reduced by as much as 50%) compared to the regular feed formulation with the 1-bin method with no influence on formula cost. Formulating feeds with stochastic programming models show how formula costs to meet the minimum nutrient specification change and the CP variability is reduced when the 2-bin method is applied.

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APPENDIX B: WINDOWS USER-FRIENDLY FEED FORMULATION FOR POULTRY 2.0

OPTIMIZATION OF FEED FORMULATION THROUGH USING DIGESTIBLE AMINO ACIDS AND TRUE PROTEIN VALUES [1]

[1] Alhotan, R. A. and G. M. Pesti. To be submitted to the Georgia Agricultural Experiment Station, Athens.

What is Windows User-Friendly Feed Formulation 2.0?

Windows User-Friendly Feed Formulation 2.0 or WUFFDA 2.0 is the first major revision of the least- cost feed formulation workbook "WUFFDA 1.0" which was published on 2004 (WUFFDA, 2004). The previous version was developed to formulate poultry and swine feeds to meet the minimum specifications of many nutrients such as crude protein (**CP**) and total amino acids (NRC, 1994 & 1998). In the current version, feed formulation is done on the basis of digestible amino acids (d AA). The workbook includes two d AA databases: one was obtained from cecectomized rooster assays (**CR**) and the other, the standardized ileal amino acid digestibility assay (**SID**), was obtained using chicks. The nutritional recommendations were obtained from commercial breeders (Aviagen, Cobb, Hy-Line). In addition, a new constraint was set to meet the minimum specification of true protein (**TP**) to provide adequate non-essential amino acids.

Why use digestible amino acids and true protein in feed formulation?

In recent years, there has been a growing interest in formulating poultry feeds using digestible amino acid values. The use of digestible amino acids can reduce dietary CP content leading to maximizing the efficiency of amino acids in protein synthesis through reducing the oxidation of the excess amino acids, maximizing profits and minimizing environmental pollution. Typically, feed is formulated to meet the requirements of essential amino acids (**EAA**) without taking into account the requirements of the non-essential amino acids (**NEAA**) or more precisely the requirements of amino nitrogen. In fact, both EAA and NEAA must be used in protein synthesis as building blocks to produce body proteins. Any deficiencies in the NEAA can lead to a significant reduction in performance; therefore, a sufficient amount of the NEAA must be assured during feed formulation. CP may be used to represent the EAA and NEAA portions but the use of CP is problematic due to the fact that part of the nitrogen content of feed comes from other non-protein nitrogen compounds (**NPN**) and not all proteins contain 16% nitrogen. On the contrary, the use of TP, which is the sum of amino acids residues composing feed proteins, can better represent the EAA plus NEAA proportion during feed formulation.

How is true protein calculated?

The minimum specification of TP in the "Nutrients" worksheet is calculated by dividing the minimum specification of dLys by a value of ~ 0.0492. This value is the TP coefficient in the linear regression equation "dLys=0.466+ 0.0492*TP" modeling the linear relationship between the dLys requirements for maximum weight gain of broilers and the TP contents of the diets fed in a meta-analysis study (Alhotan and Pesti, unpublished). Since the probability that the slope of the line (0.0492) is equal to zero is less than 0.05 and the probability that the intercept (0.466) is equal to zero is greater than 0.05, the slope of the line can be omitted from the equation and as a result TP = dLys/0.0492. Moreover, knowing that dLys is 4.92 % of TP can give an estimation of the dLys requirement when the TP value is known.

What does WUFFF DA 2.0 workbook consist of?

The WUFFDA 2.0 workbook consists of nine worksheets include Title, Ingredient, Nutrients, Sensitivity Report, Formulate, Feed Spec, Mixing Sheet, Graphs, and Coefficients worksheets (Figure 1). The 'Ingredients' worksheet contains two sections, the Active and the Storage Ingredient Composition Matrices. The abbreviation [CR] at the end of feed ingredient names indicates that the digestibility coefficients used to calculate the digestible amino acid values are based on the cecectomized rooster assay (Ajinomoto Heartland, 2015). The abbreviation [SID] refers to values from the standardized ileal amino acid digestibility assay

(Evonik, 2015). For maximum performance, the SID values are better used when formulating broiler feeds while the CR values can be used for layers and breeders. The ‘Nutrients’ worksheet contains stored nutrient specifications for broilers, broiler breeders and layers which must be selected and pasted into the current specification box to formulate feed. The digestible amino acid values are displayed as a % of the diet (the upper stored specifications) or as ratios to dLys (the lower stored specifications). The ‘Formulate’ worksheet contains two separate boxes; one box is for the ingredient composition of the complete feed and the other one for supplied nutrients. On the upper right part of the workbook there is a command button labeled “Formulate Now” that can be clicked to give a solution for the problem. The button “Get Sensitivity Report” produces a new Sensitivity report of the current formulation (posted in the Sensitivity Report’ worksheet). The ‘Feed Spec’ worksheet provides the current results of the formulation. The ‘Mixing Sheet’ worksheet displays the outputs of the current formulation in a format that facilitates adding quantities to a mixer. The supplied nutrients of the current formulation are graphed on the ‘Graph’ worksheet as proportions to the minimum requirements to easily view how levels in the solution compare to nutrient minimums. The last worksheet “Coefficients” contains the digestibility coefficients for seven essential amino acids for the cecectomized rooster assay (Ajinomoto Heartland, 2015) labeled as [CR] and the standardized ileal amino acid digestibility assay (Evonik, 2015) labeled as [SID]. This worksheet also contains the total AA contents of feed ingredients (Batal & Dale, 2015) and the coefficients used to calculate the TP values, total nitrogen contents and ingredient-specific N: P conversion factor or Ka ((Mosse, 1990; Mariotti *et al.*, 2008; Sriperm *et al.*, 2011).

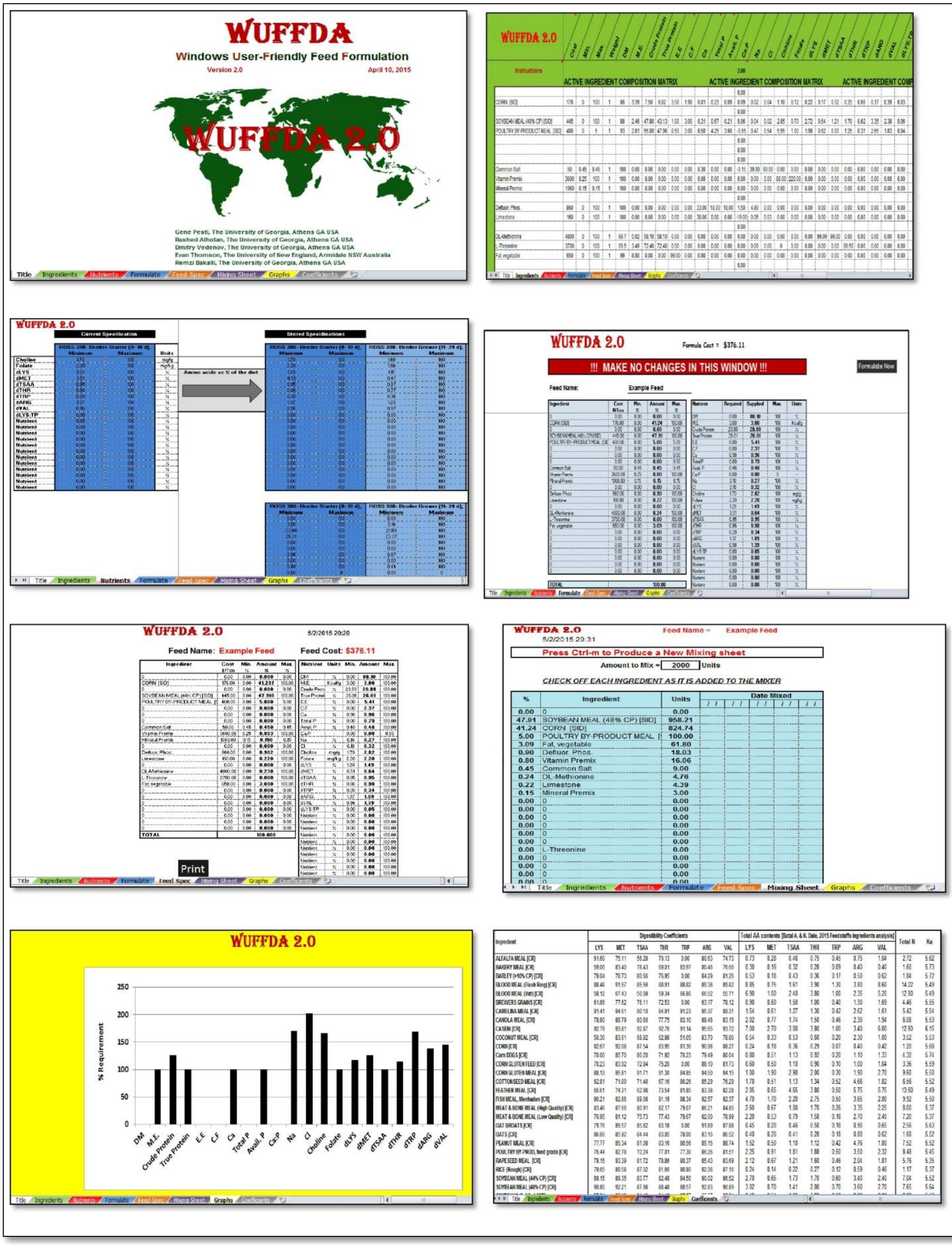


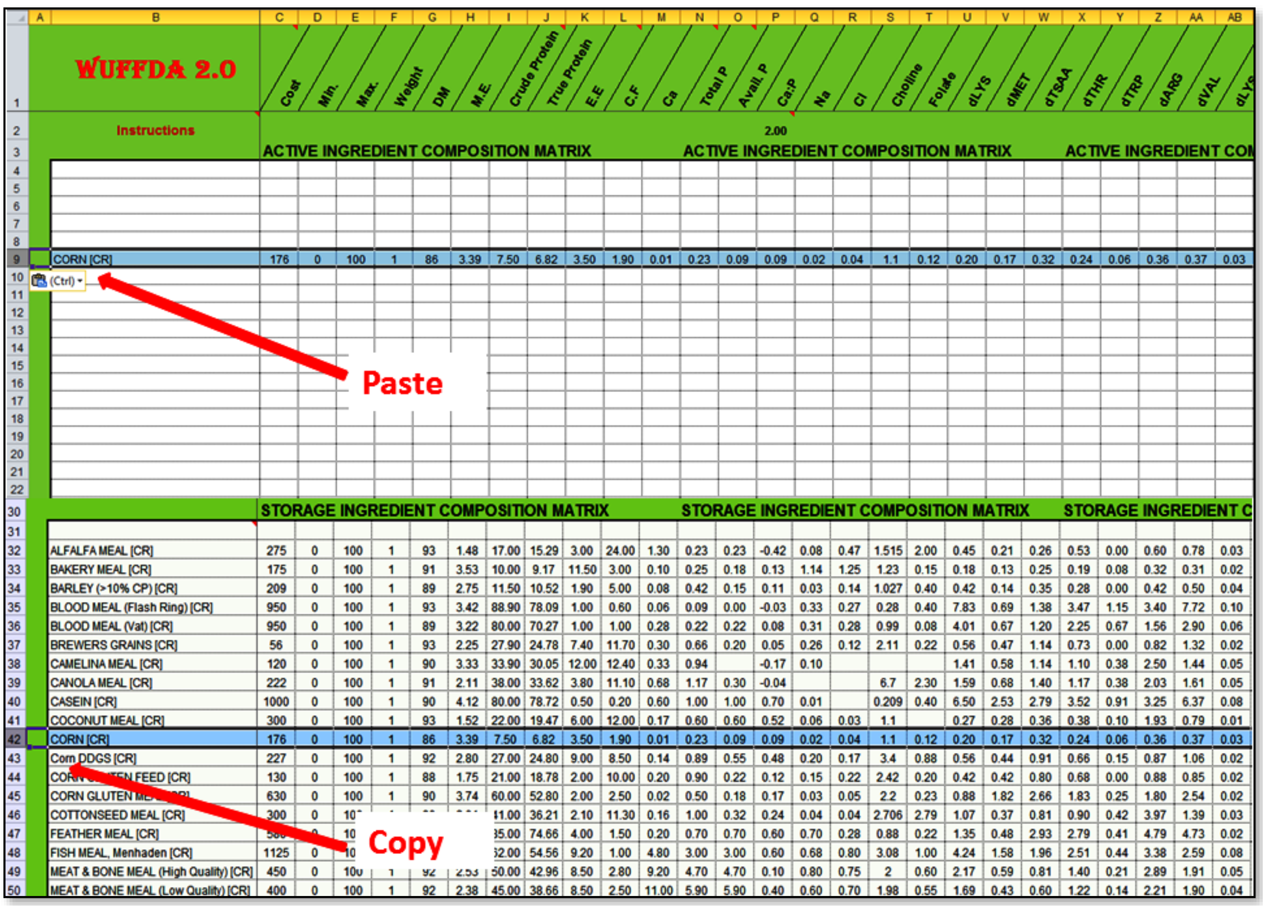
Figure 1. Overview of the worksheets of the WUFFDA 2.0 workbook

How is WUFFDA 2.0 used to formulate feed?

Feed can be formulated using WUFFDA 2.0 by following these steps:

STEP 1: Feed ingredients selection "Ingredients Worksheet"

- Copy ingredient composition data from the Storage Ingredient Composition Matrix and paste data on the Active Ingredient Composition Matrix (Figure 2).
- Data can also be entered directly.
- Remember to never delete or insert a new row or column in the active Ingredient Composition Matrix.



Row	B	Cost	Min.	Max.	Weight	DM	M.E	Crude Protein	True Protein	E.E	C.F	Ca	Total P	Avail. P	Ca:P	Na	Cl	Choline	Folate	dLYS	dMET	dTSAA	dTHR	dTRP	dARG	dVAL	dLYS
2	Instructions														2.00												
3		ACTIVE INGREDIENT COMPOSITION MATRIX											ACTIVE INGREDIENT COMPOSITION MATRIX										ACTIVE INGREDIENT COM				
9	CORN [CR]	176	0	100	1	86	3.39	7.50	6.82	3.50	1.90	0.01	0.23	0.09	0.09	0.02	0.04	1.1	0.12	0.20	0.17	0.32	0.24	0.06	0.36	0.37	0.03
30		STORAGE INGREDIENT COMPOSITION MATRIX											STORAGE INGREDIENT COMPOSITION MATRIX										STORAGE INGREDIENT C				
32	ALFALFA MEAL [CR]	275	0	100	1	93	1.48	17.00	15.29	3.00	24.00	1.30	0.23	0.23	-0.42	0.08	0.47	1.515	2.00	0.45	0.21	0.26	0.53	0.00	0.60	0.78	0.03
33	BAKERY MEAL [CR]	175	0	100	1	91	3.53	10.00	9.17	11.50	3.00	0.10	0.25	0.18	0.13	1.14	1.25	1.23	0.15	0.18	0.13	0.25	0.19	0.08	0.32	0.31	0.02
34	BARLEY (>10% CP) [CR]	209	0	100	1	89	2.75	11.50	10.52	1.90	5.00	0.08	0.42	0.15	0.11	0.03	0.14	1.027	0.40	0.42	0.14	0.35	0.28	0.00	0.42	0.50	0.04
35	BLOOD MEAL (Flash Ring) [CR]	950	0	100	1	93	3.42	88.90	78.09	1.00	0.60	0.06	0.09	0.00	-0.03	0.33	0.27	0.28	0.40	7.83	0.69	1.38	3.47	1.15	3.40	7.72	0.10
36	BLOOD MEAL (Vat) [CR]	950	0	100	1	89	3.22	80.00	70.27	1.00	1.00	0.28	0.22	0.22	0.08	0.31	0.28	0.99	0.08	4.01	0.67	1.20	2.25	0.67	1.56	2.90	0.06
37	BREWERS GRAINS [CR]	56	0	100	1	93	2.25	27.90	24.78	7.40	11.70	0.30	0.66	0.20	0.05	0.26	0.12	2.11	0.22	0.56	0.47	1.14	0.73	0.00	0.82	1.32	0.02
38	CAMELINA MEAL [CR]	120	0	100	1	90	3.33	33.90	30.05	12.00	12.40	0.33	0.94		-0.17	0.10				1.41	0.58	1.14	1.10	0.38	2.50	1.44	0.05
39	CANOLA MEAL [CR]	222	0	100	1	91	2.11	38.00	33.62	3.80	11.10	0.68	1.17	0.30	-0.04			6.7	2.30	1.59	0.68	1.40	1.17	0.38	2.03	1.61	0.05
40	CASEIN [CR]	1000	0	100	1	90	4.12	80.00	78.72	0.50	0.20	0.60	1.00	1.00	0.70	0.01		0.209	0.40	6.50	2.53	2.79	3.52	0.91	3.25	6.37	0.08
41	COCONUT MEAL [CR]	300	0	100	1	93	1.52	22.00	19.47	6.00	12.00	0.17	0.60	0.60	0.52	0.06	0.03	1.1		0.27	0.28	0.36	0.38	0.10	1.93	0.79	0.01
42	CORN [CR]	176	0	100	1	86	3.39	7.50	6.82	3.50	1.90	0.01	0.23	0.09	0.09	0.02	0.04	1.1	0.12	0.20	0.17	0.32	0.24	0.06	0.36	0.37	0.03
43	Corn DDGS [CR]	227	0	100	1	92	2.80	27.00	24.80	9.00	8.50	0.14	0.89	0.55	0.48	0.20	0.17	3.4	0.88	0.56	0.44	0.91	0.66	0.15	0.87	1.06	0.02
44	COR[illegible]EN FEED [CR]	130	0	100	1	88	1.75	21.00	18.78	2.00	10.00	0.20	0.90	0.22	0.12	0.15	0.22	2.42	0.20	0.42	0.42	0.80	0.68	0.00	0.88	0.85	0.02
45	CORN GLUTEN ME[illegible]	630	0	100	1	90	3.74	60.00	52.80	2.00	2.50	0.02	0.50	0.18	0.17	0.03	0.05	2.2	0.23	0.88	1.82	2.66	1.83	0.25	1.80	2.54	0.02
46	COTTONSEED MEAL [CR]	300	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]1.00	36.21	2.10	11.30	0.16	1.00	0.32	0.24	0.04	0.04	2.706	2.79	1.07	0.37	0.81	0.90	0.42	3.97	1.39	0.03
47	FEATHER MEAL [CR]	[illegible]	[illegible]	10[illegible]	[illegible]	[illegible]	[illegible]	[illegible]5.00	74.66	4.00	1.50	0.20	0.70	0.70	0.60	0.70	0.28	0.88	0.22	1.35	0.48	2.93	2.79	0.41	4.79	4.73	0.02
48	FISH MEAL, Menhaden [CR]	1125	0	10[illegible]	[illegible]	[illegible]	[illegible]	[illegible]2.00	54.56	9.20	1.00	4.80	3.00	3.00	0.60	0.68	0.80	3.08	1.00	4.24	1.58	1.96	2.51	0.44	3.38	2.59	0.08
49	MEAT & BONE MEAL (High Quality) [CR]	450	0	100	1	92	2.53	50.00	42.96	8.50	2.80	9.20	4.70	4.70	0.10	0.80	0.75	2	0.60	2.17	0.59	0.81	1.40	0.21	2.89	1.91	0.05
50	MEAT & BONE MEAL (Low Quality) [CR]	400	0	100	1	92	2.38	45.00	38.66	8.50	2.50	11.00	5.90	5.90	0.40	0.60	0.70	1.98	0.55	1.69	0.43	0.60	1.22	0.14	2.21	1.90	0.04

Figure 2. 'Ingredient' worksheet showing the selection of feed ingredients

STEP 2: Nutritional requirements selection “Nutrients Worksheet”

- Highlight the desired nutrient specification from the Stored Specifications section and click on the right mouse button to copy the selection (Figure 3).
- Place the cursor in cell “C4” and paste the selected cells.
- To change the amino acid profile, select the desired nutrient specification from the lower boxes (amino acids referenced to dLys). Enter the new dLys value the corresponding cell and then re-calculate the sheet to update the values.

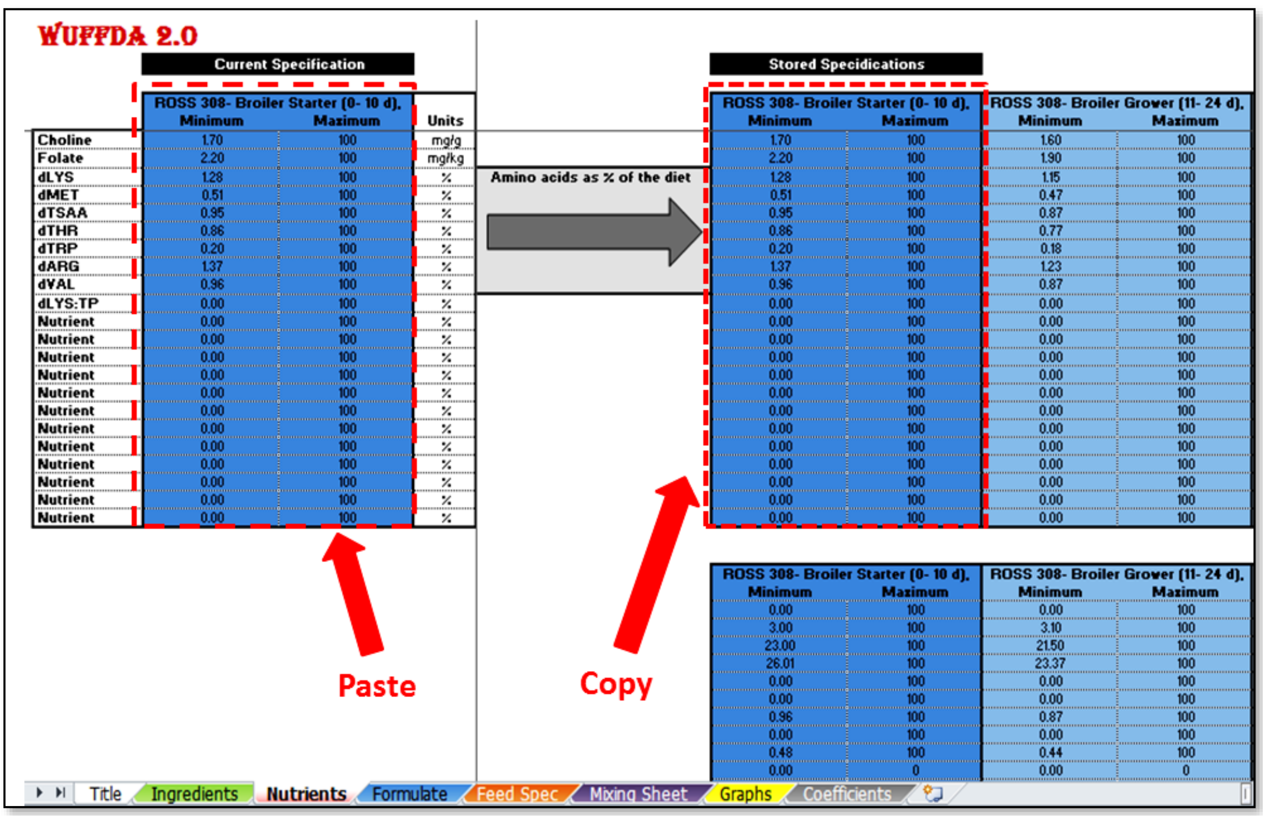
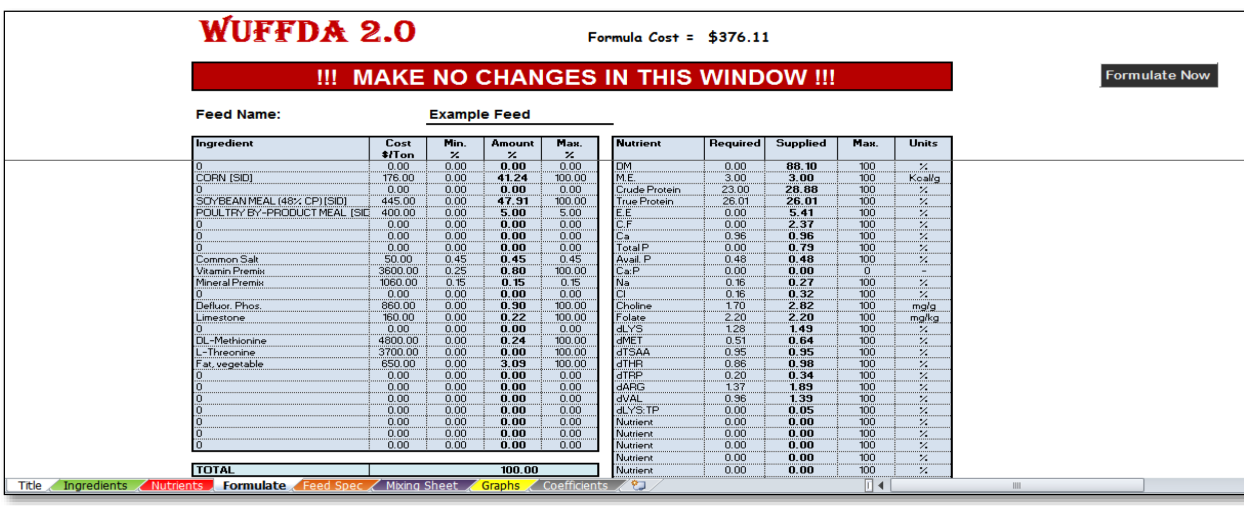


Figure 3. Nutritional requirements selection in the ‘Nutrients’ worksheet

STEP 3: Formulate the diet “Formulate Worksheet”

- Feed can be formulated by simply clicking on the “Formulate Now” button located in the upper right of the worksheet (Figure 4) or by choosing “Solver” From the Tools Menu to solve the feed formulation problem.
- The ingredient composition of the feed is displayed in the “Amounts” column on the left side of the worksheet, and the nutrients supplied by those amounts is displayed on the right side in the “Supplied” column.
- Note that the required levels of all nutrients including true protein in the current feed formulation problem have been met (true protein=26.01). In this example the requirements are for Ross 308 starting broilers.
- When no feasible solution is found one or more values are displayed in red. The constraints should be relaxed one at a time until a feasible solution is displayed. By relaxing the constraints this way the problem can usually be identified easily.


WUFFDA 2.0

Formula Cost = $376.11

!!! MAKE NO CHANGES IN THIS WINDOW !!!

Formulate Now

Feed Name: Example Feed

Ingredient	Cost $/Ton	Min. %	Amount %	Max. %
0	0.00	0.00	**0.00**	0.00
CORN [SID]	176.00	0.00	**41.24**	100.00
0	0.00	0.00	**0.00**	0.00
SOYBEAN MEAL (48% CP) [SID]	445.00	0.00	**47.91**	100.00
POULTRY BY-PRODUCT MEAL [SID	400.00	0.00	**5.00**	5.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
Common Salt	50.00	0.45	**0.45**	0.45
Vitamin Premix	3600.00	0.25	**0.80**	100.00
Mineral Premix	1060.00	0.15	**0.15**	0.15
0	0.00	0.00	**0.00**	0.00
Defluor. Phos.	860.00	0.00	**0.90**	100.00
Limestone	160.00	0.00	**0.22**	100.00
0	0.00	0.00	**0.00**	0.00
DL-Methionine	4800.00	0.00	**0.24**	100.00
L-Threonine	3700.00	0.00	**0.00**	100.00
Fat, vegetable	650.00	0.00	**3.09**	100.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
0	0.00	0.00	**0.00**	0.00
TOTAL			**100.00**	

Nutrient	Required	Supplied	Max.	Units
DM	0.00	**88.10**	100	%
M.E.	3.00	**3.00**	100	Kcal/g
Crude Protein	23.00	**28.88**	100	%
True Protein	26.01	**26.01**	100	%
E.E	0.00	**5.41**	100	%
C.F	0.00	**2.37**	100	%
Ca	0.96	**0.96**	100	%
Total P	0.00	**0.79**	100	%
Avail. P	0.48	**0.48**	100	%
Ca:P	0.00	**0.00**	0	-
Na	0.16	**0.27**	100	%
Cl	0.16	**0.32**	100	%
Choline	1.70	**2.82**	100	mg/g
Folate	2.20	**2.20**	100	mg/kg
dLYS	1.28	**1.49**	100	%
dMET	0.51	**0.64**	100	%
dTSAA	0.95	**0.95**	100	%
dTHR	0.86	**0.98**	100	%
dTRP	0.20	**0.34**	100	%
dARG	1.37	**1.89**	100	%
dVAL	0.96	**1.39**	100	%
dLYS:TP	0.00	**0.05**	100	%
Nutrient	0.00	**0.00**	100	%
Nutrient	0.00	**0.00**	100	%
Nutrient	0.00	**0.00**	100	%
Nutrient	0.00	**0.00**	100	%
Nutrient	0.00	**0.00**	100	%

Figure 4. The ‘Formulate’ worksheet showing the results of the current feed formulation problem

STEP 4: Review the feed formulation results.

- Go to the "Graphs" worksheet to view the supplied nutrients as proportions to the minimum requirements (Figure 5).

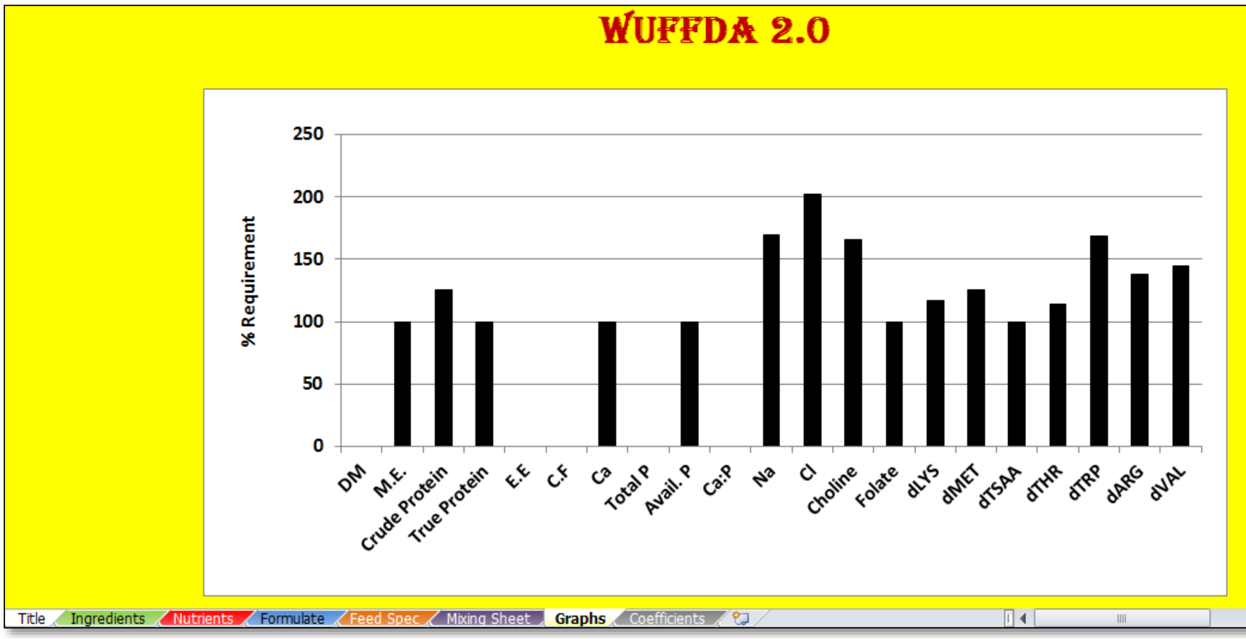


Figure 5. The supplied nutrients presented as proportions of the minimum requirements.

What can be concluded from WUFFDA 2.0?

WUFFDA 2.0 provides a feature to account for the needs of the NEAA by satisfying the minimum specification of TP. TP should be representative of all amino acids in the feed when an appropriate minimum level is set for it. When the minimum level of TP was set to zero in the current formulation problem (Ross 308 starting broilers), the supplied TP and CP levels were ~ 21% and 23%, respectively. When the minimum level of TP was set to 26.01% (as a function of dLys), the supplied TP and CP levels were ~ 26% and 29%, respectively. The increase in protein should satisfy the needs for the NEAA or amino nitrogen. Formula cost increased from $316 per ton of feed to $355 since more SBM and less corn were used to supply the increased level of protein. The decision to use the TP levels in feed formulation should be based on the costs of the inputs and the value of the final product.

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APPENDIX C: MAXIMUM INGREDIENT LEVEL OPTIMIZATION WORKBOOK FOR ESTIMATING THE MAXIMUM SAFE LEVELS OF FEEDSTUFFS [1]

[1] Alhotan, R. A., D. V. Vedenov and G. M. Pesti. To be submitted to the Georgia Agricultural Experiment Station, Athens.

SUMMARY

New feed ingredients are evaluated and introduced to the feed industry every year. The evaluation process is necessary and includes feeding birds different levels of the test ingredient to estimate the maximum safe level (MSL). The MSL is estimated usually with a multiple range test ignoring the fact that this test is inappropriate for this type of feeding trials where the independent variable is continuous. This paper describes the use of the Maximum Ingredient Optimization Workbook (MIOW) in estimating the MSL and determining the optimal combination of ingredient levels and replications for most efficient experimental design of future feeding trials. The MIOW workbook calculates the results and the related descriptive statistics (SD, SE, CI, and R^2) based on simulation and non-linear regression models (broken-line linear and broken-line quadratic models).

Why is Estimating the Maximum Safe Level of a Feed Ingredient Important?

Potential feedstuffs are being evaluated every year as new feed ingredients for livestock. The evaluation process includes feeding the test ingredients at increasing levels to groups of birds or animals and then the pattern of the biological response and/or the maximum safe level of this ingredient can be estimated. The biological response of feeding an ingredient varies depending on several factors for instance, the age of the bird, species or the chemical composition of the ingredient. One scenario that reflects a response to an ingredient in a feeding trial (Gamboa *et al*., 2001) is illustrated in Figure 1. Feeding increasing levels of cottonseed meal had no impact on the growth performance of broilers (up to a certain point) as represented by the plateau segment of the curve. Further increase in the cottonseed meal level resulted in reducing the growth performance as represented by the descending segment of the line. Underestimating the maximum safe level of cottonseed meal will not maximize the economic returns of including this ingredient in the ration while overestimating the level of the meal will result in a significant reduction in growth performance due to the nature of the chemical composition of the ingredient (e.g. high levels of antinutritional factors). Therefore, finding the maximum safe level of feed ingredients precisely is required to maximize the performance and the profits.

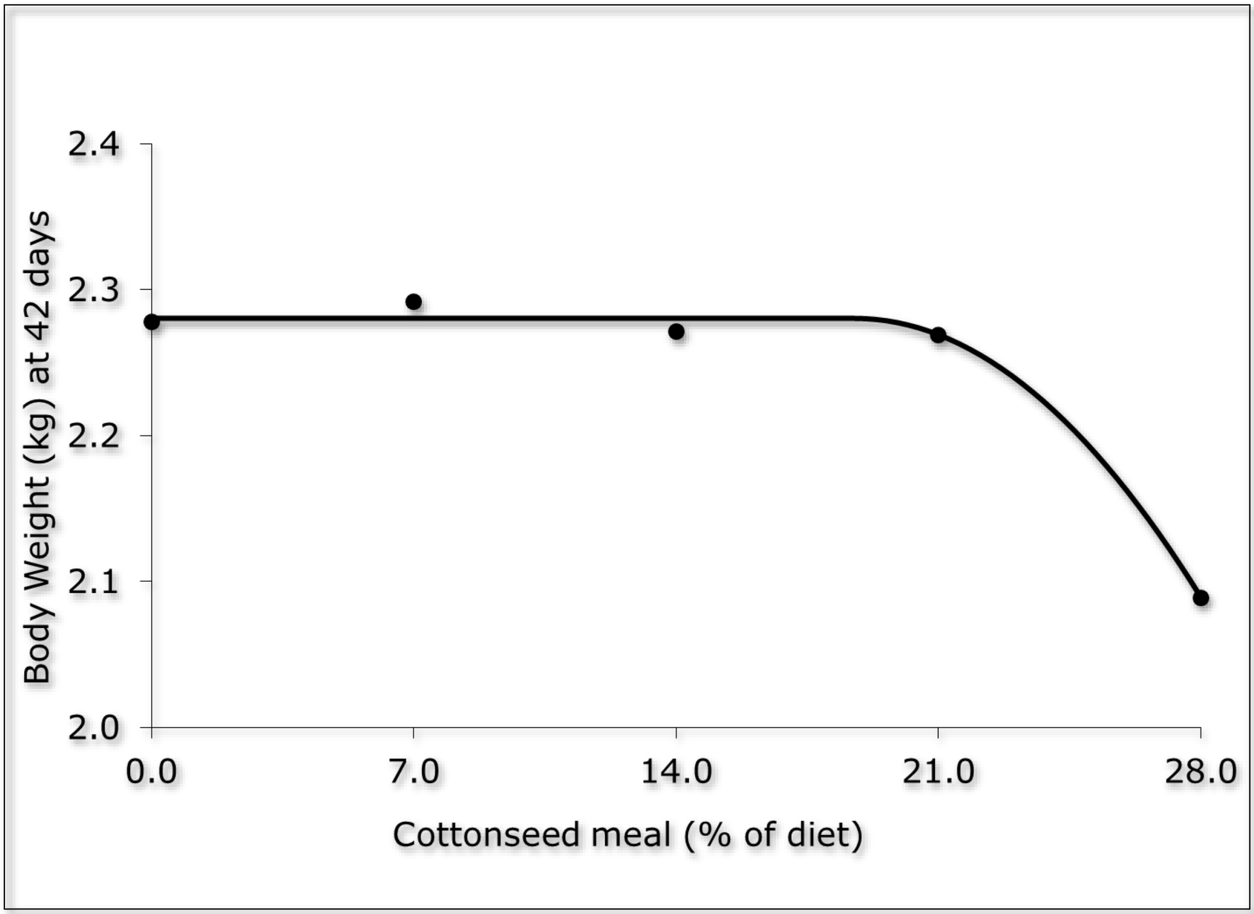

Figure 1. Growth response of broiler chickens fed increasing levels of cottonseed meal (Gamboa et al., 2001)

What are the Statistical Methods Used to Estimate the Maximum Safe Level of Feedstuffs?

Several statistical methods have been used in animal feeding trials to estimate the maximum safe level of feed ingredients. One common and easy method is separating means of the response variable using multiple range tests. The multiple range tests are based on one-way analysis of variance and are designed for categorical data to distinguish between the levels of the factor. The maximum safe level is defined here as the maximum level of the feed ingredient that results in a response that is not significantly different from the maximum or minimum response at a chosen level of significance. These tests may not be valid to analyze data obtained from feeding trials where the factor is continuous since 1) the actual safe level can only be on or between two levels; 2) More conservative tests (e.g. Scheffe's test, 1953 vs. Duncan's test, 1955) will result in detecting fewer significant differences and 3) Extrapolation and constructing confidence interval for a mean are not possible in this case. Another method used in the feeding trials is the orthogonal contrasts which compares levels against the control group. Since fewer numbers of comparisons are made, this method is more precise than the multiple range tests but they are not really orthogonal and distinguish only between levels as with the multiple range tests. Employing regression analysis helped to understand the pattern of the data (e.g. linear, quadratic, etc.). The factor is treated here as a continuous variable and the maximum safe level is determined by finding the 1st derivative (maximum level may be underestimated). However, the regression models used provide no feature to fit the plateau segment of the curve.

What Does the Maximum Ingredient Level Optimization Workbook Do?

The maximum ingredient level optimization workbook or MIOW (Microsoft Corp., Redmond, WA) estimates the maximum safe level of feed ingredients and the related descriptive statistics (confidence interval (CI), standard deviation (SD), standard error (SE) and the R^2 for the fitted relationship) by two spline functions and the calculations are based on a series of simulated experiments. The response to increasing levels of feed ingredients is modeled by three mathematical models; broken-line linear model (BLL), broken-line quadratic model (BLQ) and second-order polynomial (2OP) model. The BLL model depicts a constant response to increasing levels of the ingredient (plateau with a slope of zero) followed by a linear response (descending line). The BLQ model depicts a constant response to increasing levels of the ingredient (plateau with a slope of zero) then a non-linear diminishing returns response (descending line). The 2OP depicts a non-linear diminishing returns response.

What Does The Maximum Ingredient Level Optimization Workbook Consist Of?

The MIOW workbook contains several individual worksheets (Figure 2): Home page, Instructions, Levels & Reps, Simulations and Calculations worksheets. The Levels & Reps worksheet is designed to generate an experimental grid (levels & replications combinations) for the experiment being simulated. The experimental grid contains the levels of the ingredient as well as the number of replications of the experiment. The simulations worksheet contains sections for the entry of the true parameters of the response function, initial guesses for the parameters of fitted functions, simulation parameters, results and a graph of the results.

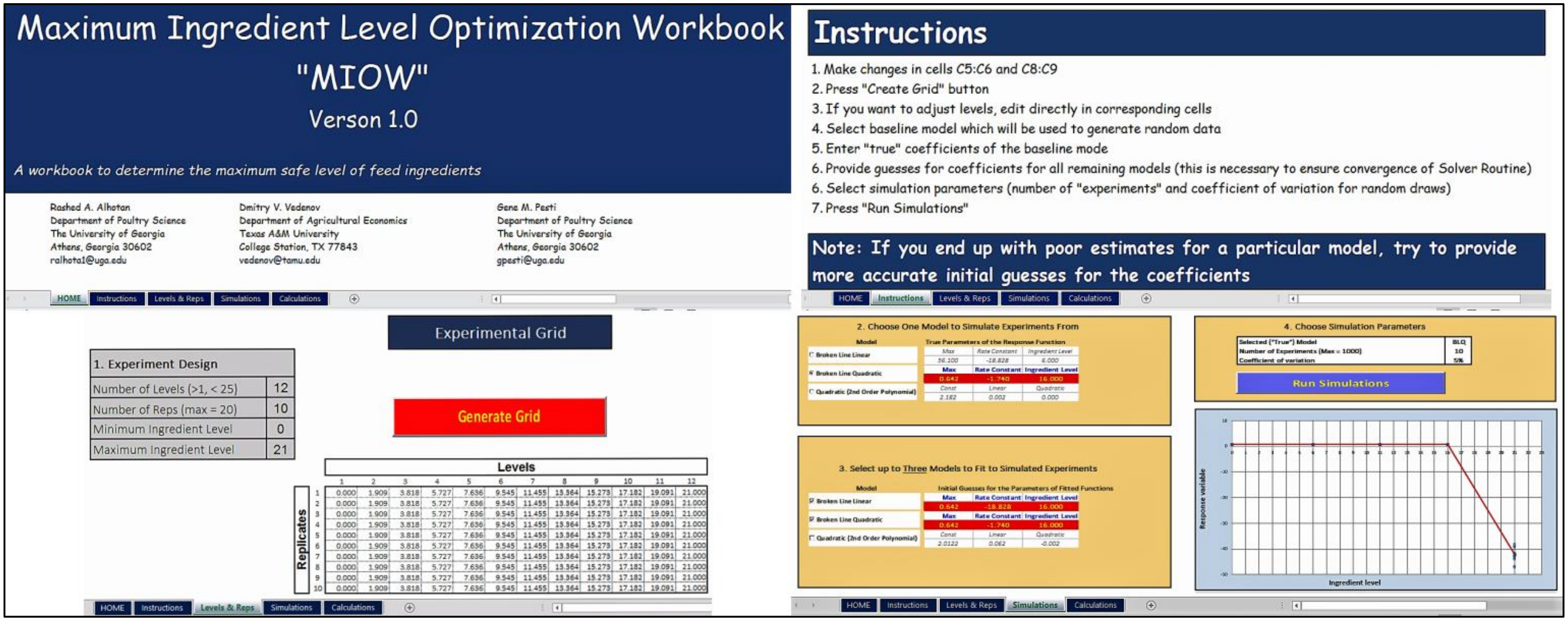

Figure 2. Overview of the maximum ingredient level optimization workbook worksheets

How is the Maximum Ingredient Level Optimization Workbook Used?

The MIOW workbook can be used by following the next steps:

1. Design the experiment being simulated by making changes in cells C5:C6 and C8:C9. The number of ingredient levels and the number of replications of the experiment being simulated can be entered in cells C5 and C6, respectively. It should be noted that the maximum number of levels that can be used here is limited to 24 while the maximum number of replicates is 20. The minimum and maximum ingredient levels should be entered in cells C8 and C9, respectively.
2. Press "Generate Grid" button to create the experimental grid.

   By clicking on "Generate Grid" button a table containing the experimental replicates and the associated ingredient levels will be created. The levels of the ingredient being used will be evenly spaced. In a feeding trial (Moghaddam *et al*, 2012), four levels of Sunflower meal (0-21%) were used and each dietary treatment was replicated 4 times. Figure 3 shows the experimental grid after updating the experimental design section with the experimental design information from this research. The data contained in the table will be used in the 'Calculations' worksheet in model fitting.

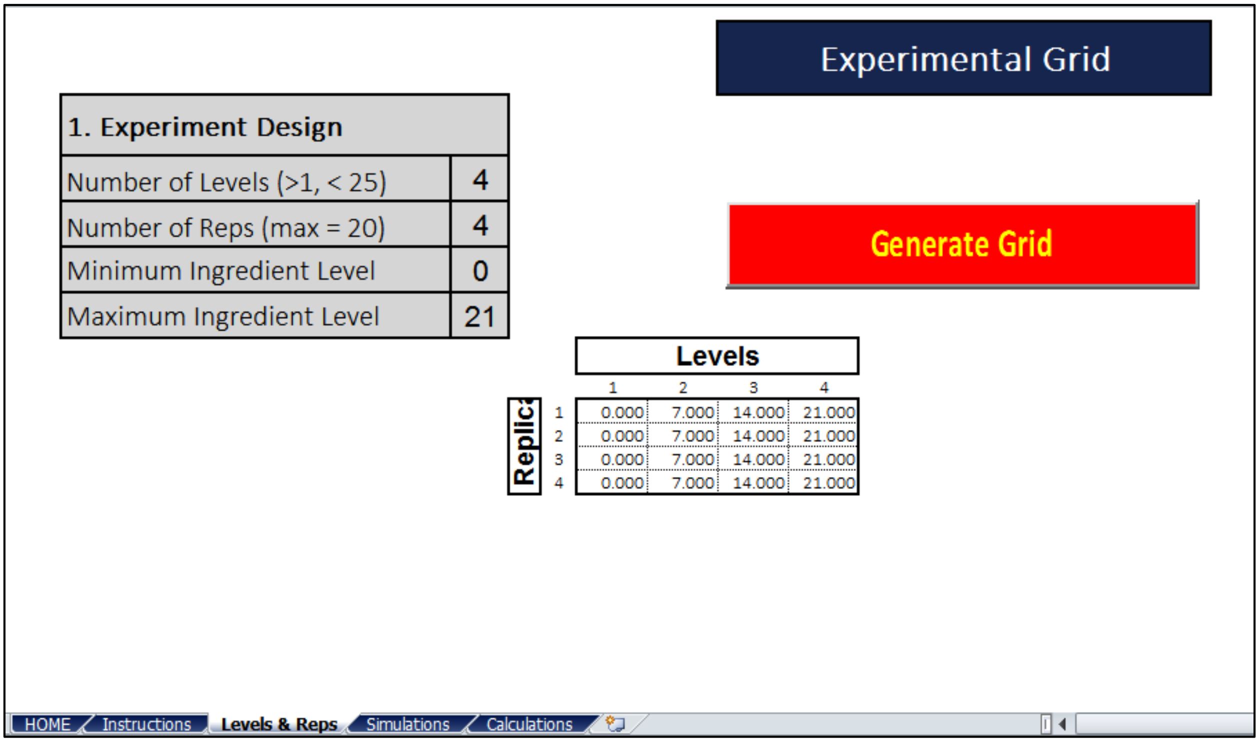

Figure 3. Experimental grid generation- Part of the 'Levels & Reps' Worksheet

3. Select baseline model which will be used to generate random data.

   Three mathematical models are available to select from in section two of the 'Simulations' worksheet (Figure 4). The models are Broken-Line Linear, Broken-Line quadratic and 2nd order polynomial. Only one model can be selected at a time to simulate experiments.

4. Enter "true" coefficients of the baseline mode

   The maximum value of the response variable (e.g. weight gain), the rate constant of the fitted function and the level of the ingredient producing the maximum response should be entered in the corresponding cells for each of the broken line models. The true parameters of the 2nd order polynomial of the form of $y= \beta_o+\beta_1 x+\beta_2 x^2+\epsilon$ include constant term (β_o), linear term (β_1) and quadratic term (β_2) and should be entered in the specified cells. In the example (Moghaddam *et al*, 2012), the maximum weight gain at 49 days was 2.472 kg for the group of chicken fed 14% sunflower meal. Section two of the ‘Simulation’ worksheet was updated with these values as true coefficients. The true coefficients will be used to generate random data using simulation.

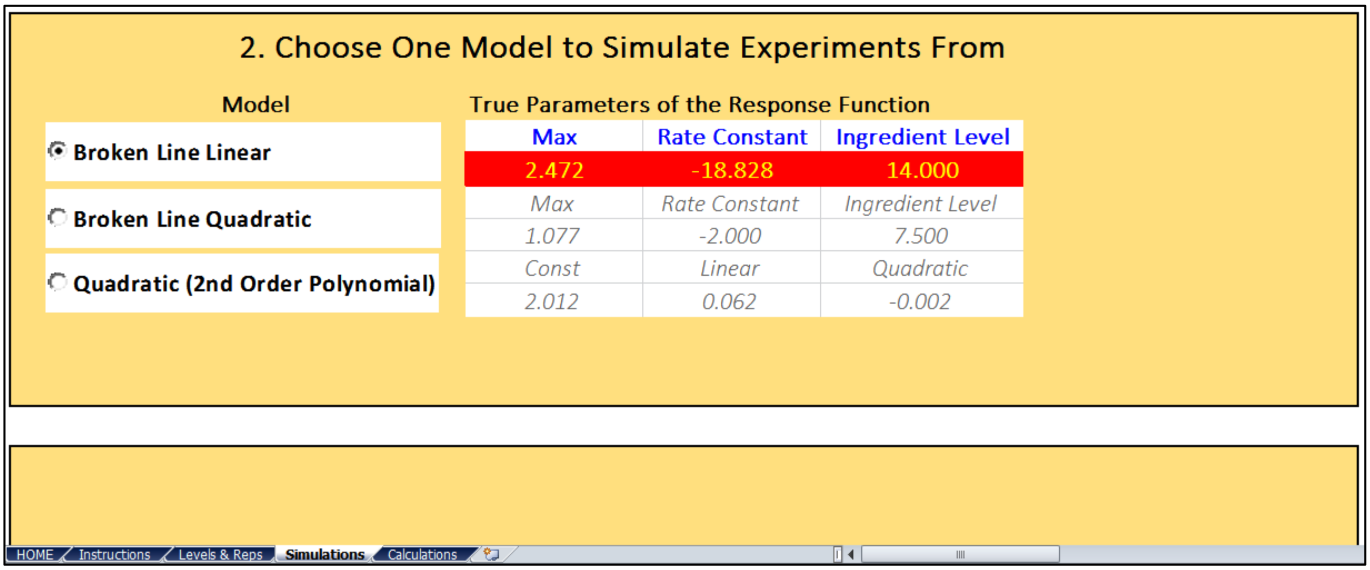

Figure 4. Baseline model selection- Part of the ‘Simulation’ Worksheet

5. Provide guesses for coefficients for all remaining models.

   Initial guesses for the regression coefficients of each model as in Figure 5 should be entered to ensure convergence of solver routine. The rate constant should be a negative value if the second part of the curve is descending (e.g. weight gain).

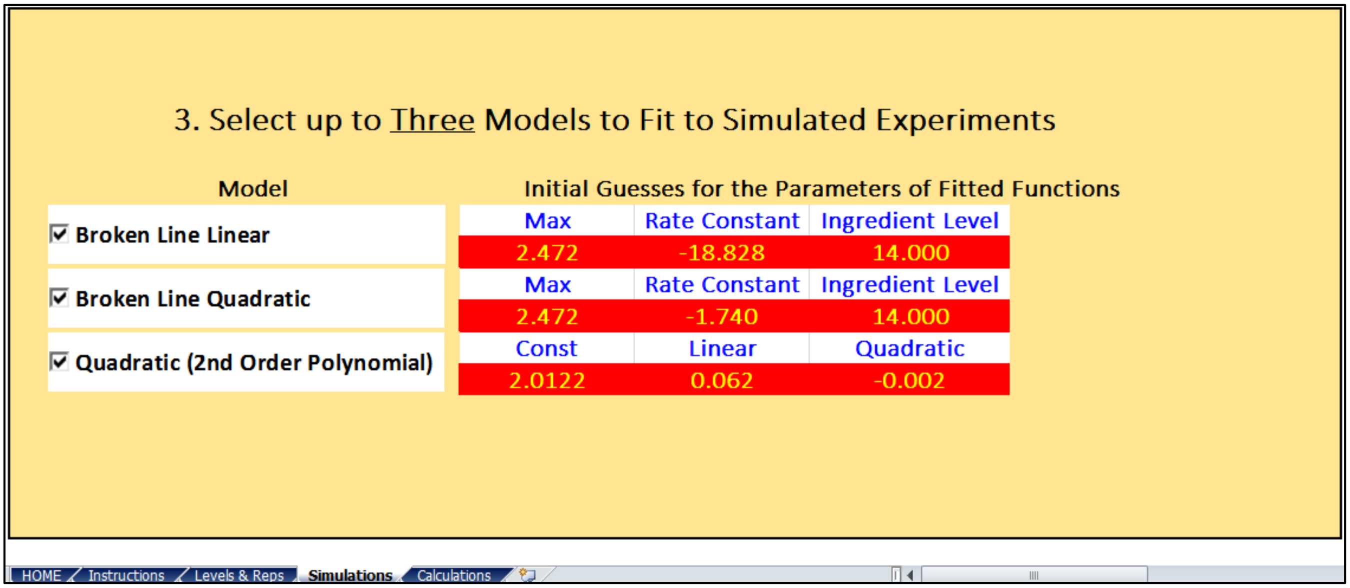

Figure 5. Initial guesses and model selection- Part of the 'Simulation' Worksheet

6. Select simulation parameters.

The number of experiments to be simulated and coefficient of variation (CV) for the simulated experiments should be provided in cells O6 and O7, respectively. Table 1 suggests that a minimum of 50 simulated experiments is enough (based on minimizing SD and SE) when estimating the MSL with broken-line models. To reflect the real life situations, a certain amount of variability must exist in the simulated experiments. Table 2 shows that as the coefficient of variability (CV) increases the SD and SE increase accordingly and the goodness of fit (R^2) decreases. A number of 100 simulated experiments and a CV value of 10% were chosen in the current simulation example to ensure minimum SD and SE and high R^2.

Table 1. Effect of increasing the number of simulated experiments on estimating the maximum safe level of Sunflower meal by broken-line models at a fixed CV of 10%, ingredient level of 5 and replication number of 4

N[1]	Broken-Line Linear						Broken-Line Quadratic					
	MSL (%)[2]	± SD	± SE	95% Confidence		R^2	MSL (%)[2]	± SD	± SE	95% Confidence		R^2
				Lower	Upper					Lower	Upper	
2	13.91	0.06	0.29	13.83	13.99	0.98	10.30	0.13	0.65	10.12	10.48	0.98
10	13.96	0.22	0.21	13.83	14.09	0.99	10.42	0.48	0.48	10.12	10.72	0.99
50	13.97	0.17	0.21	13.93	14.02	0.99	10.44	0.39	0.48	10.34	10.55	0.99
100	13.99	0.16	0.22	13.96	14.02	0.99	10.48	0.35	0.49	10.41	10.55	0.99
500	14.01	0.17	0.20	14.00	14.03	0.99	10.53	0.37	0.45	10.50	10.56	0.99
1000	13.99	0.16	0.20	13.98	14.00	0.99	10.48	0.36	0.46	10.46	10.50	0.99

[1] **Number of simulated experiments**
[3] **Maximum safe level of the test ingredient**

Table 2. Effect of increasing variation of the simulated experiments on estimating the maximum safe level of Sunflower meal by broken-line models with 100 simulated experiments of 5 levels and 4 replications

CV (%)[1]	Broken-Line Linear						Broken-Line Quadratic					
	MSL (%)[2]	± SD	± SE	95% Confidence		R^2	MSL (%)[2]	± SD	± SE	95% Confidence		R^2
				Lower	Upper					Lower	Upper	
0	14.00	0.00	0.00	NA[3]	NA	1.00	10.50	0.00	0.00	10.50	10.50	1.00
5	14.00	0.08	0.11	13.99	14.02	1.00	10.50	0.19	0.24	10.47	10.54	1.00
10	13.98	0.14	0.21	13.96	14.01	0.99	10.47	0.31	0.48	10.40	10.53	0.99
20	13.97	0.34	0.41	13.91	14.04	0.96	10.46	0.73	0.93	10.31	10.60	0.96
50	13.82	1.13	NA	13.60	14.05	0.79	9.90	1.73	2.72	9.56	10.24	0.80
100	14.62	2.43	NA	14.15	15.10	0.51	9.18	6.04	NA	7.99	10.36	0.51

[1] **Coefficient of Variation**
[2] **Maximum safe level of the test ingredient**
[3] **Not estimated**

7. Press "Run Simulations".

   This option when clicked will optimize the simulation problem producing a graph (Figure 7) and the results of the simulation (Figure 8).

4. Choose Simulation Parameters

Selected ("True") Model	BL
Number of Experiments (Max = 1000)	100
Coefficient of variation	10%

Run Simulations

Figure 6. Simulation parameters selection and the "Run" button- Part of the "Simulation" Worksheet

How to Read the Results?

The results of the simulation problem for the current example (Moghaddam *et al*, 2012) are displayed in section five of the 'Simulation' worksheet (Figure 8). The descriptive statistics displayed in rows 37:40 are the results of the 100 experiments simulated for each model. For the BLL model, the maximum safe level of the sunflower meal as an average for the 100 simulated experiments (runs) -/+ SD was 14.005% ± 0.125 (95% CI = 13.981- 14.029%) for an estimated maximum weight gain of 2.475 ± 0.032 kg. The SE of the maximum safe level was calculated to be 0.091. The R^2 of the fitted BLL model function was estimated to be 98.8% which implies a good fit. Similarly, the results of the BLQ model are displayed in columns J to N of section 5. For the 2OP model, the calculated maximum safe level was 6.618 ± 0.054. The estimated regression coefficients were -14.146, 8.639 and – 0.653 as the constant, linear term and the quadratic term, respectively. Poor estimates of the results for any model may require more accurate guesses of the coefficients as they influence the goodness of fit.

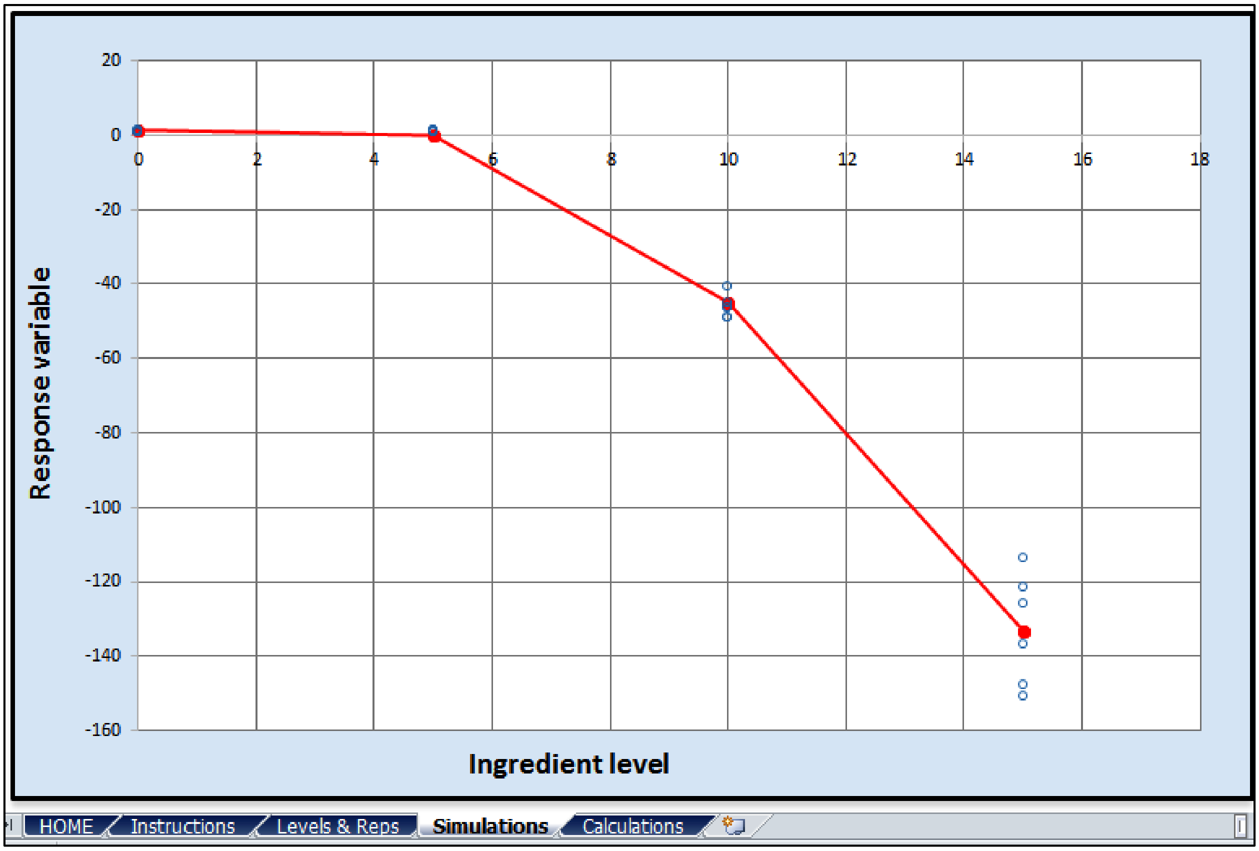

Figure 7. Graph of the results - Part of the “Simulation” Worksheet

Experiment #	Model Fits														
	Broken Line Model					Broken Line Quadratic Model					Quadratic (2nd Order Polynomial)				
	Max/Min	Rate Constant	MSL	R^2	SE of MSL	Max/Min	Rate Constant	MSL	R^2	SE of MSL	Const	Linear	Quadratic	MSL (Calculated)	R^2
Average	2.475	-18.912	14.005	98.8%	0.091	3.173	-1.380	10.892	98.0%	0.236	-14.146	8.639	-0.653	6.618	92.8%
Std. Dev.	0.032	0.850	0.125	0.4%	0.018	0.107	0.105	0.248	0.6%	0.038	0.791	0.337	0.021	0.054	0.4%
Lower 95%	2.469	-19.078	13.981	98.7%	0.088	3.152	-1.401	10.844	97.8%	0.229	-14.301	8.573	-0.657	6.608	92.7%
Upper 95%	2.481	-18.745	14.029	98.9%	0.095	3.194	-1.359	10.941	98.1%	0.244	-13.991	8.705	-0.648	6.629	92.9%
0001	2.4813	-19.5549	14.0536	99.0506%	0.082	3.1589	-1.4425	10.9881	98.3713%	0.210	-14.7114	8.9138	-0.6715	6.6376	93.0585%
0002	2.4914	-19.7111	14.0870	99.0078%	0.083	3.1545	-1.4611	11.0379	98.2894%	0.214	-14.8545	8.9627	-0.6737	6.6518	92.8402%
0003	2.5208	-20.6189	14.2348	97.5306%	0.124	2.9514	-1.6410	11.4493	97.6550%	0.242	-15.8326	9.3435	-0.6943	6.7284	91.3292%
0004	2.4390	-18.7790	13.9674	98.1883%	0.117	3.1533	-1.3571	10.8291	97.2999%	0.276	-14.1041	8.6121	-0.6518	6.6061	92.3440%
0005	2.5177	-18.0122	13.8887	98.4823%	0.110	3.2613	-1.2791	10.6879	97.3933%	0.275	-13.3305	8.2973	-0.6311	6.5740	92.7275%
0006	2.4902	-18.7123	13.8386	98.0715%	0.127	3.3243	-1.3089	10.5802	96.7851%	0.309	-13.9248	8.6322	-0.6589	6.5508	92.3331%
0007	2.4533	-18.9139	14.0539	99.2631%	0.072	3.0895	-1.3984	10.9989	98.5592%	0.197	-14.1855	8.6186	-0.6492	6.6382	93.1641%
0008	2.5041	-18.0617	13.9338	98.9227%	0.091	3.2070	-1.2968	10.7734	97.9345%	0.242	-13.4763	8.3179	-0.6302	6.5989	93.1163%
0009	2.5185	-19.2978	14.0872	98.8849%	0.088	3.0725	-1.4619	11.1267	98.5364%	0.196	-14.5979	8.8148	-0.6615	6.6630	92.9115%
0010	2.5719	-17.8975	13.8065	98.7172%	0.104	3.4607	-1.2280	10.4685	97.1720%	0.293	-13.0344	8.2494	-0.6316	6.5306	92.9263%
0011	2.4541	-17.5316	13.8039	98.1790%	0.124	3.3023	-1.2096	10.4899	96.7562%	0.313	-12.8772	8.0954	-0.6195	6.5337	92.4889%
0012	2.4708	-18.5332	13.9258	98.8172%	0.096	3.2056	-1.3281	10.7577	97.8524%	0.247	-13.8190	8.5145	-0.6463	6.5870	93.0112%
0013	2.4921	-19.7694	14.0909	98.7072%	0.094	3.2230	-1.4485	10.9921	97.8621%	0.241	-14.8047	8.9633	-0.6744	6.6456	92.5251%
0014	2.4551	-20.5105	14.2077	98.9046%	0.083	3.0443	-1.5721	11.2766	98.5070%	0.195	-15.5337	9.2409	-0.6900	6.6966	92.4956%
0015	2.4725	-18.5394	13.9235	99.1665%	0.080	3.1565	-1.3422	10.7993	98.3451%	0.216	-13.9739	8.5571	-0.6484	6.5987	93.4332%
0016	2.5243	-19.1706	14.1356	98.8193%	0.089	3.1489	-1.4342	11.1169	98.1740%	0.220	-14.3047	8.6799	-0.6508	6.6682	92.5526%
0017	2.4516	-18.2511	13.8705	99.2893%	0.075	3.2751	-1.2769	10.6080	97.9945%	0.242	-13.4089	8.3683	-0.6388	6.5504	93.4237%
0018	2.4521	-18.2121	13.9600	99.1333%	0.081	3.2227	-1.2939	10.7502	97.9876%	0.239	-13.5593	8.3464	-0.6323	6.6004	93.1972%
0019	2.4657	-19.3381	14.0532	99.2971%	0.070	3.1989	-1.4061	10.9283	98.3651%	0.211	-14.4780	8.7964	-0.6631	6.6326	93.1842%
0020	2.4335	-18.9436	13.9855	98.4075%	0.109	3.1399	-1.3750	10.8628	97.5676%	0.261	-14.1245	8.6484	-0.6549	6.6027	92.5153%
0021	2.4582	-18.8659	14.0081	99.1912%	0.077	3.1687	-1.3690	10.8812	98.3010%	0.217	-14.1264	8.6217	-0.6513	6.6193	93.2213%

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Figure 8. The results section- Part of the “Simulation” Worksheet

What are Other Uses of the Maximum Ingredient Level Optimization Workbook?

The MIOW Workbook can also be used to decide the best combinations of the ingredient levels and replications when designing feeding trials. The combination with the smallest SE of the MSL mean should be the most efficient combination. As the replication number increased from 2 to 20 the SE of the MSL decreased for both models (Table 3). The SE of the MSL couldn't be estimated with 4 levels and a minimum of 5 levels was required for the estimation of the SE under the conditions (true parameters and initial guesses) of the current simulation example (Table 4).

Table 3. Simulations of increasing the number of replications on estimating the maximum safe level of Sunflower meal by broken-line models. Based on 100 simulated experiments with a CV of 10% and 5 ingredient levels

N[1]	Broken-Line Linear					Broken-Line Quadratic				
	MSL (%)[3]	± SD	± SE	95% Confidence		MSL (%)[3]	± SD	± SE	95% Confidence	
				Lower	Upper				Lower	Upper
1	13.94	0.42	0.02	13.85	14.02	10.39	0.86	0.09	10.23	10.56
2	14.00	0.24	0.23	13.95	14.04	10.49	0.55	0.51	10.39	10.60
4	14.00	0.15	0.21	13.97	14.03	10.50	0.34	0.47	10.43	10.57
6	13.96	0.13	0.19	13.94	13.99	10.42	0.30	0.43	10.36	10.48
8	14.00	0.11	0.15	13.98	14.02	10.50	0.24	0.35	10.45	10.55
10	14.01	0.11	0.14	13.99	14.03	10.53	0.24	0.31	10.48	10.58
12	14.00	0.09	0.13	13.99	14.02	10.51	0.20	0.30	10.47	10.54
14	14.00	0.08	0.12	13.98	14.01	10.49	0.19	0.28	10.45	10.53
16	14.00	0.08	0.11	13.99	14.02	10.50	0.18	0.26	10.47	10.54
18	14.00	0.07	0.11	13.98	14.01	10.49	0.17	0.25	10.46	10.53
20	14.00	0.08	0.10	13.98	14.01	10.50	0.17	0.23	10.46	10.53

[1] **Number of replications**
[2] **Maximum safe level of the test ingredient**

Table 4. Simulations of increasing the number of Levels on estimating the maximum safe level of Sunflower meal by broken-line models. Based on 100 simulated experiments with a CV of 10% and 4 replications

N[1]	Broken-Line Linear					Broken-Line Quadratic				
	MSL (%)[2]	± SD	± SE	95% Confidence		MSL (%)[2]	± SD	± SE	95% Confidence	
				Lower	Upper				Lower	Upper
2	13.98	0.31	NA	13.92	14.04	13.49	0.13	NA	13.47	13.52
3	13.98	0.26	NA	13.93	14.04	13.49	0.12	NA	13.46	13.51
4	14.12	0.16	NA	14.09	14.15	13.90	0.11	NA	13.88	13.92
5	14.00	0.17	0.22	13.96	14.03	10.49	0.38	0.51	10.42	10.57
6	13.99	0.34	0.24	13.93	14.06	10.27	0.39	0.49	10.20	10.35
8	14.00	0.14	0.16	13.97	14.03	10.57	0.35	0.43	10.50	10.64
15	14.01	0.14	0.12	13.98	14.04	10.79	0.28	0.30	10.74	10.85
20	14.00	0.10	0.09	13.98	14.02	10.85	0.22	0.26	10.81	10.90
24	14.01	0.12	0.09	13.98	14.03	10.89	0.25	0.24	10.84	10.94

[1] Number of ingredient levels
[2] Maximum safe level of the test ingredient

What Can Be Concluded From the Maximum Ingredient Level Optimization Workbook?

The MIOW workbook offers a method to estimate the maximum safe level of test ingredients and the related statistics (CI, SD, SE and R^2). Unlike the multiple range and the orthogonal contrast approaches, the broken-line and the quadratic polynomial models of the MIOW Workbook treat the independent variable as continues and offer estimations of the descriptive statistics of the means. The SD provides information on the dispersion of the data while the SE tells how accurate the estimate of the mean is. The goodness of fit as represented by the R^2 should help users to determine how well the model fits the data. The MIOW Workbook can be used to find the best combination of levels and replications when designing feeding trials. The combination with the smallest SD and SE should be the most efficient design.

Computational Requirement

All modern versions of Microsoft Excel with Visual Basic function should be enough to run the MIOW Workbook. Macros need to be enabled before using the workbook.

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