Assessing the Response of Laying Hens to Digestible Balanced Protein from 27 to 66 Weeks of Age

A Thesis

Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in the Department of Animal and Poultry Science,

University of Saskatchewan,

Saskatoon, SK,

Canada.

By

Dinesh Kumar

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon, Saskatchewan S7N 5A8

ABSTRACT

As laying hens continue to improve in egg characteristics, it is relevant to examine their response to dietary digestible balanced protein (DBP). Research using Lohmann-LSL hens, from 27 to 66 wk of age, compared DBP intake levels using diets with essential amino acids (AA) balanced to digestible lysine (Dlys) intake levels of 550, 625, 700, 775 and 850 mg/hen/day. Each treatment was replicated 10 times with 12 birds per replicate. Two base diets containing 500 or 850 mg of Dlys per 100 g of diet were blended every three wk based on previous feed intakes to produce diets that would provide treatment levels of Dlys intake. Data collection included daily hen-day egg production (HDEP), and feed intake (FI), egg weight (EW), egg mass (EM), feed efficiency (FE) and egg specific gravity (ESG) every three wk. Hens were weighed and scored for feather cover at 27, 47 and 66 wk of age. Egg components were measured at 41, 52 and 65 wk of age, including albumen height (AH), dry egg shell weight (ESW), and albumen and yolk weights (AW and YW). At the end of the experiment, pectoralis muscle (PM), abdominal fat pad (AFP), heart, liver and kidneys were collected from 10 randomly euthanized hens per treatment and weighed. Empty weight and length of gastrointestinal tract (GIT) segments were also measured. Excreta samples were collected twice from five randomly selected cages per treatment to determine total excreta nitrogen (N) content. In a completely randomized experimental design, the data were analyzed using one-way ANOVA as mixed model and by using PROC regression and PROC response surface regression procedures of SAS 9.3 (2003). Differences in means were considered significant when $P \leq 0.05$. The effect of increasing DBP on hen weight, HDEP, EW, EM, FI and FE was quadratic. By using a non-linear regression model, a maximum response for HDEP, EW, EM and FE was observed at 769, 836, 903 and 839 mg/h/d intake of Dlys, respectively. Increasing Dlys levels increased AFP weight linearly and

PM weight increased quadratically. Heart, liver and kidney weights were not affected by Dlys intake. Proventriculus absolute weight was increased while gizzard weight decreased linearly with increasing Dlys intakes. Intestinal segment weights decreased linearly or quadratically with increasing Dlys intake. Caeca measurements were not affected by the treatment. Feather score increased with Dlys intake level. Both percent ESW and ESG decreased linearly with increasing intake levels of Dlys. Absolute AW (linear) and YW (quadratic) increased with Dlys intake; proportional values for these criteria were affected in a quadratic manner, but the effects were small. Excreta N content increased in a quadratic manner with increasing amino acid intake. In conclusion, the response of Lohmann-LSL hens to DBP was determined and the DBP (based on diets with essential AA balanced to Dlys) level required to maximize the response varied with the criteria being assessed.

Keywords: ideal protein, egg mass, body composition, plumage, egg quality, excreta nitrogen

ACKNOWLEDGMENTS

With a full sense of respect, I would like to extend my sincere gratitude and appreciation to my supervisor, Dr. Henry L. Classen for accepting me as his student. His adept guidance, timely support and advice to my queries throughout the course of this project was outstanding. I would like to thank my graduate committee members; Dr. Tom Scott and Dr. Karen Schwean-Lardner for their valuable support and guidance. I would like to thank Dr. Martin Zuidhof for his valuable time, useful comments, and serving as an external examiner.

I am thankful to Natural Sciences and Engineering Research Council of Canada – Industry Research Chair (NSERC-IRC) in Poultry Nutrition and its following sponsor organizations: Chicken Farmers of Saskatchewan, Saskatchewan Egg Producers, Saskatchewan Turkey Producers, Saskatchewan Hatching Egg Producers, Sofina Foods Inc., Prairie Pride Natural Foods Ltd., Poultry Industry Council, Canadian Poultry Research Council, and Aviagen for providing financial support for this project.

Thanks to Dawn Abbott for her technical assistance and for arranging the logistics of data collection. I would like to acknowledge the special contribution of Robert Gonda for his assistance in preparing the experiment diets. I would also like to thank Centaine Raginski, University of Saskatchewan Poultry Centre staff and fellow graduate students for their help and support.

My special thanks to Shelley Revering, Feed Director at Federated Co-operatives Ltd., for allowing me to pursue this degree while working full-time with this organization. Last but not least, the love and support from my family and friends was astounding during this period.

Dedication

I would like to dedicate this thesis to my family

for

their wonderful love and affection

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVATIONS

AA	Amino acid/s
AH	Albumen height
AW	Albumen weight
BCAA	Branched chain amino acid/s
BP	Balanced protein
BW	Body weight
DBP	Dietary balanced protein
Dlys	Digestible lysine
EAA	Essential amino acids
ESG	Egg specific gravity
EW	Egg weight
FE	Feed efficiency
FI	Feed intake
FS	Feather Score
HDEP	Hen day egg production
HHEP	Hen housed egg production
HW	Hen weight
L	Linear
LE	Lysine efficiency
LNAA	Large neutral amino acids
ME	Metabolizable energy
mg/h/d	milligram per hen per day
NEAA	Non-essential amino acids
Q	Quadratic
TFD	True fecal digestible
wk	Week/s
YW	Yolk weight

1. INTRODUCTION

Eggs have been considered as one of the cheapest source of animal based food protein for human consumption. Laying hens have been selected extensively in the last five decades and their egg production and egg quality have continuously increased. In 1958, the brown and white-shell hens laid 214 and 212 hen-housed eggs, respectively, compared to 281 and 276 in 2009 (Anderson *et al.*, 2013) in 500 days of age. With reduced hen weight, feed intake has decreased and feed efficiency has increased because of a lower nutrient requirement for maintenance. The continuous increase in egg mass production increases the absolute nutrient requirement of hens and the reduced feed intake (increased feed efficiency) means increased nutrient content must be present at higher proportional levels in the diet. This has encouraged research to estimate the hen's nutrient (energy, protein, AA etc.) requirements for growth, maintenance and egg production. An ideally balanced nutrition, especially for protein and AA, is critical for the expression of the full genetic potential of these birds. The increasing cost of diet protein and increasing pressure from environmental groups regarding environmental pollution from nitrogen and ammonia has challenged poultry nutritionists to create diets which minimize nitrogen excretion while still meeting the amino acid requirements of birds.

Protein and AA are considered key nutrients in laying hen diets to attain the desired egg production and feed efficiency (Bregendahl *et al.*, 2008). Clearly, hens do not have a demand for protein *per se.* But, to synthesize body proteins for growth and maintenance, and also for egg proteins, hens have a dietary requirement for AA. Generally, hens require 20 amino acids to synthesize body and egg proteins. Among them, 10 are essential amino acids (EAA), or dietary indispensable AA which are: arginine, lysine, leucine, isoleucine, valine, threonine, tryptophan,

phenylalanine, histidine and methionine. The other 10 are non-essential amino acids (NEAA), or dietary dispensable AA and namely they are: glycine, serine, alanine, cysteine, proline, aspartic acid, asparagine, glutamic acid, glutamine and tyrosine. The lower the gap between the hen's AA requirement and the dietary AA composition, the better the utilization of the dietary proteins. Research has been conducted to estimate the ideal EAA requirements of laying hens (Coon and Jhang, 1999; Bonekamp *et al.*, 2010), but the results have not been consistent. Further, little research has examined the role of NEAA in poultry diets, particularly when diets are formulated to contain low CP (Kerr and Kidd, 1999; Bregendahl *et al*., 2002). The National Research Council (NRC, 1994) of United States and the Centraal Veevoederbureau of Netherlands (CVB – Central Bureau for Livestock Feeding, 1996) in Europe set guidelines for EAA and other key nutrients as ideal requirements for laying hens and other poultry species.

In commonly used soybean meal and corn or wheat based poultry diets, methionine is the first limiting AA followed by lysine and threonine. According to the ideal AA profile concept, dietary lysine has been considered more significant and the levels of other EAA are formulated relative to lysine level. Lysine has high metabolic importance and its deficiency can result in a reduction in weight and egg performance. Maximum growth rate or egg production can be achieved by meeting minimum AA requirements, but birds fed these diets tend to not utilize feed efficiently. A higher level of balanced AA dietary profile is needed to maximize the efficiency of feed utilization (Wu *et al*., 2007).

A daily lysine intake recommendation of 690 mg/h/d by the NRC (1994) for laying hens is not considered adequate as suggested by recent studies and recommendations from commercial breeding companies. The objective of the current study was to assess the response of laying hens to graded levels of digestible lysine intake (balanced for essential AA) from 27 to 66 wk of age

on egg production, feed utilization, egg components, hen weight and body composition, feathering, and excreta total nitrogen content. It was hypothesized that the digestible DBP requirement of laying hens is higher than current recommendations and is also dependent on the response criteria being assessed.

2. LITERATURE REVIEW

2.1. A general overview of protein and amino acids

2.1.1. Proteins

Proteins are complex organic molecules and are considered as building blocks of life (micro-organisms, plants, animals and humans). They are biologically essential for building body structure and for other critical functions of body tissues. Proteins can also provide energy when required, as they contain 4 kcal of energy g^{-1}. Like other nutrients, proteins are also important components of organisms and perform vital cellular functions. Structurally, proteins are made of amino acids. Amino acids are bound to each other by peptide linkages to produce polymers termed peptides. Each protein consists of at least one polypeptide. Proteins differ from each other by the number and length of polypeptides they contain, and also by the sequence of their amino acids. The biological and functional properties of proteins are affected by the number and the sequencing pattern of AA in the primary polypeptide chain. Dietary protein sources supply amino acids and nitrogen that are essential for synthesis of meat and egg proteins (Shim *et al*., 2013; van Emous *et al*., 2013). Proteins also perform other important functions in the animal's body such as enzymes to assist in metabolic reactions, building and maintaining of cytoskeletons (collagen, elastin, actin, keratin etc.), hormones to perform vital physiological functions, and cell signalling in an immune response (Tan and Parker, 2003).

2.1.2. Protein digestion

Dietary proteins are large and complex molecules, and are unable to be absorbed directly from the gut. As described by Jurgens (2002), upon ingestion, dietary proteins are first denatured by hydrochloric acid (HCl) in the gastric stomach and their peptide linkages are exposed to enzymatic actions. The HCl also converts the inactive pepsinogen endopeptidase into pepsin

enzyme. Under the low pH (1.5 - 2.0) of the gastric stomach, pepsin performs the initial break-down of proteins into smaller peptides. Then in the small intestine, these smaller peptides are further hydrolysed into free amino acids by the enzymatic action of pancreatic endopeptidases such as trypsin, chymotrypsin, elastase and carboxypeptidase, and other intestinal enzymes like aminopeptidase, dipeptidase and tripeptidase. Free amino acids and dipeptides are released in the digestive tract and are absorbed through the intestinal wall into the blood circulation (Silk *et al.*, 1985). These hydrolysis products are actively absorbed in the duodenum and jejunum and then the absorption process is slowed down in the ileum. The digested amino acids and peptides are mainly absorbed from the intestines via two mechanisms; carrier proteins transport system and glutathione transport system. Free amino acids in the diet are directly absorbed, mainly from the upper small intestine (Wu, 2009). The absorbed amino acids are then added to the amino acid pool of the body and are utilized for synthesis of proteins for various purposes such as tissue growth, maintenance and reproduction.

2.1.3. Amino acids

Amino acids are organic compounds and are the building units of proteins. As explained by Hardy (1985), the key components of an AA are carbon (C), hydrogen (H), oxygen (O) and nitrogen (N) atoms. Structurally, an AA consists of a two-carbon bond; one carbon is a part of the carboxyl group (COO^-), which consists of one C and two O atoms, and the other C is linked to an amino group. Amino stands for a bonding of an NH_2 group to a C atom. There are hundreds of amino acids which have been identified. However, only 20 amino acids are considered important to synthesize protein in animals and humans, and they are called natural or proteinogenic AA. The body cannot synthesize all of the 20 amino acids. The amino acids which

the body cannot synthesize are called ‘essential or indispensable amino acids’ and those synthesized by the body are known as ‘non-essential or dispensable amino acids’.

2.2. Protein and amino acids for poultry

Along with other nutrients, an adequate supply of dietary protein and AA is required for birds to achieve their maximum performance. The dietary supplied protein and AA are utilized by birds for growth (structural tissues, muscle etc.), maintenance (tissues, feathers etc.) and reproduction (egg production). Synthesis and degradation of body proteins is a continuous process. An adequate availability of amino acids, and also in their ideal ratios, is necessary to support this process. Dietary protein provides nitrogen (N) to synthesize non-essential AA. The quality of a protein relies on the arrangement of its amino acids. Different methodologies such as apparent/standardized/true ileal or fecal digestibility, can be applied to determine the bio-availability of these amino acids in poultry diets. The sources of poultry dietary protein can be animal-based (meat meal, meat and bone meal, fish meal) and or plant-based (soybean meal, canola meal, peas).

In laying hens and other poultry classes, AA nutrition plays a vital role in achieving high levels of performance (Emmert and Baker, 1997; Bregendahl *et al*., 2008). However, there are several other factors, in addition to ideal nutrition, which can also influence the performance of poultry such as bird genotype, age, health status, feed intake and management. These factors, in addition to rate of egg production and rate of growth, can influence their AA requirements. Dietary energy level in poultry diets should be adequate to ensure maximum utilization of dietary protein and AA. Feed to egg mass conversion ratio was linearly reduced when dietary ME was gradually increased from 2,719 to 2,956 kcal/kg of diet in Dekalb White hens from 21 to 36 wk of age (Wu *et al*., 2007). Possibly due to sparing of EAA usage for energy purposes.

2.2.1. Essential amino acids (EAA) for poultry

Poultry species are incapable of making all of the amino acids that are essential to synthesize proteins for growth and reproduction. There are 10 essential amino acids in poultry and they are: arginine and lysine (base AA), and leucine, isoleucine and valine (branched-chain AA), threonine, tryptophan, phenylalanine, histidine, and methionine (sulfated AA). Essential amino acids are supplied to birds via dietary protein sources and synthetic AA. Many *in-vivo* experiments have been conducted to evaluate the ideal requirement of these EAA for different poultry species to obtain maximum growth and production responses, and also for efficient feed utilization (Hurwitz *et al.*, 1983; Emmert and Baker, 1997; Bregendahl *et al.*, 2008). In poultry, methionine is the first limiting amino acid in commonly used soybean meal and corn or wheat based diets, followed by lysine, threonine, tryptophan and isoleucine. NRC (1994) published recommendations for the required supply of dietary protein, EAA and other important nutrients for poultry. The amino acid requirements can be influenced by four important factors, which are strain, the environment, bird health status and dietary factors (Ishibashi, 1990). The requirement for essential amino acids changes with bird age and stage of production. These days, many EAA are also available in synthetic or crystalline form and are considered to be 100% digestible. The levels of essential amino acids are carefully balanced in poultry diets so that they complement each other's functions. Lysine has been recognized as a reference AA and all other EAA levels are adjusted relative to dietary lysine level.

2.2.2. Non-essential amino acids (NEAA)

Non-essential amino acids can be synthesized by animals including birds. Namely, they are alanine, glycine, serine, cysteine, tyrosine, glutamine, glutamate, aspartic acid, aspartate and asparagine. Birds use blood nitrogen derived from metabolism of dietary protein to synthesize

these NEAA. Poultry diets should contain enough crude protein to provide nitrogen for synthesis of these NEAA. Generally, low crude-protein diets balanced with EAA have not been very successful (Pur and Samie, 2007). The provision of easily synthesized NEAA such as glutamic and aspartic acid to increase crude protein has also been of negligible value (Kerr and Kidd, 1999; Bregendahl *et al.,* 2002). However, supplementing poultry diets with glycine and/or serine have been found to be beneficial (Dean *et al*., 2006; Waguespack *et al*., 2009; Berres *et al*., 2010). Egg performance was reduced with low-protein diets supplemented with EAA and that might be due to low levels of other NEAA (Penz and Jensen, 1991). With too much of a reduction in dietary protein, even with sufficient supplementation with EAA, a situation may arise that some of the EAA are diverted in the synthesis of NEAA due to the lack of non-specific nitrogen (Heger, 2003). This situation may harm the performance of the birds by reducing the body protein deposition and egg production.

2.2.3. Digestibility of amino acids

Birds obtain their amino acids from dietary protein sources and also from dietary supplemented amino acids. All the amino acids consumed are not necessarily 100% digested. There are many factors which can influence the amino acid digestibility such as the dietary source of amino acids, environmental conditions, management, and genetics, age and health status of the animal. Today, we know the digestibility values (apparent, standard or true) for most essential amino acids in different feed ingredients (Lemme *et al.,* 2004). Modern poultry diet formulations are based on the digestible AA values of feed ingredients instead of total dietary values. Performance prediction can be more accurately made when diets fed are ideally balanced on digestible values of the amino acids. The AA digestibility of feed ingredients can be

significantly improved with application of exogenous enzymes and also by applying appropriate feed processing techniques (Newkirk and Classen, 1998).

2.2.4. Amino acid imbalance, interaction, antagonism and toxicity

Undesired effects occur if one of the limiting amino acid is supplemented at a much higher level than the requirement in relation to the levels of one or more EAA in the group. This condition may result in reduced feed intake and performance (Peganova and Eder, 2002). Reduced feed consumption resulting in low intake of limiting amino acids is mainly responsible for low bird performance. Such adverse effects can be avoided by supplementing the most limiting amino acid in the diet. The inappropriate balance of amino acids can be classified as being related to imbalance, antagonistic and toxic effects (Harper, 1964). A diet with adequate dietary protein may produce amino acid imbalance characteristics if the diet is supplemented with one or a group of indispensable amino acids (Calderon and Jensen, 1990; Davis and Austic, 1994). Branched chain amino acids (BCAA) have been considered detrimental to poultry and other species if they are consumed in excess amounts (Tuttle and Balloun, 1976). An interaction among structurally similar BCAA (leucine, isoleucine and valine) has been reported when their levels were imbalanced in laying hen diets (Peganova and Eder, 2003). In the latter experiment, feed consumption and egg production were significantly reduced when the dietary isoleucine was increased from 5.7 mg to 11.5 mg/100 g of the diet. Harper (1958) also reported low plasma levels of valine and isoleucine, and reduced feed intake and growth, when rats were fed low protein diet (9% CP) supplemented with 10% leucine AA. The interaction between BCAA is due to sharing a common mode of cellular transportation and catabolism by a common enzyme (Harper, 1984).

An antagonism between excessive lysine level and arginine has also been well documented. (D'Mello and Lewis, 1970). Lysine and arginine share a common transport mechanism for absorption (Olszewski, 1978). Also, in chicks, excessive lysine intake increases arginase enzyme activity in the kidneys resulting in increased catabolism of arginine (Ball *et al*., 2007). An amino acid toxicity can be defined as a condition of severe growth depression resulted by excessive feeding of an individual amino acid which cannot be prevented by supplementing the diet with other amino acid or a group of amino acids. Baker (2006) suggested that growth depression caused by excessive feeding of methionine was partially reversed by supplementing the diet with glycine.

2.3. Amino acids for laying hens

In the last few decades, the egg performance characteristics in laying hens have improved while the BW has decreased (Elliott, 2008). These changes might have influenced amino acid requirements for egg production and maintenance. Amino acids are key elements of a laying hen diet. An adequate supply of balanced EAA is necessary to fulfil the hens' full genetic potential. Inherently, hens do not have a demand for protein. But hens do have a physiological requirement for amino acids for body growth and maintenance, and for synthesis of egg proteins. They also use amino acids to produce other physiologically important non-protein compounds such as serotonin, nitric oxide, adrenaline and carnitine. The protein and ideal AA requirement of commercial layers can be influenced by hen strain, age, environmental temperature, body weight and variations in feed intake (Sá *et al.,* 2007).

2.3.1. Methionine

Methionine is first limiting amino acid in most commercial laying hen diets and methionine has a major influence on egg production and egg size (Harms *et al.*, 1998). More research has

been conducted on methionine and total sulfur amino acids (TSAA, methionine + cysteine) requirements than any other amino acid (Harms and Russell, 1998; Safaa *et al.*, 2008). Egg size increases when hens grow older and the increase in egg size can be controlled by reducing the TSAA and linoleic acid in the diet (Safaa *et al*., 2008). The methionine and cysteine ratio in TSAA for laying hens has been recommended as 50:50 (NRC, 1994). However, some of the methionine can be converted into cysteine through the trans-sulfuration pathway. Cysteine plays a key role in protein synthesis by creating disulfide bonds. There is considerable variation in the estimates of daily requirements for methionine and TSAA for commercial layers. The NRC (1994) daily intake recommendation of 300 mg and 580 mg/h/d of total methionine and TSAA, respectively, is lower than what is used by commercial egg producers and recommended by most breeder companies (Lohmann-LSL management guide, 2013). Bregendahl *et al*. (2008) suggested 253 and 506 mg/h/d of true digestible methionine and TSAA, respectively, for a maximum egg mass response in Hy-line W-36 hens. In a more recent meta-analysis study by Van Krimpen *et al*. (2015), it has been suggested that the hens require 670 mg/h/d of digestible methionine and cysteine for maximum egg mass which is far higher than older studies.

2.3.2. Lysine

Lysine is an α – amino acid and a base. It is an indispensable AA in animals, poultry and humans. Lysine is the second most limiting amino acid in commonly used commercial laying hen diets and also when diets are formulated to contain minimum CP levels. In diet formulations following the ideal protein concept, the levels of all other EAA are set as relative to dietary lysine level. The daily total lysine intake recommendation of 690 mg/h/d by the NRC (1994). The CVB (1996) has recommended 700 mg/h/d of digestible lysine for laying hens, while Coon and Jhang (1999) suggested 676 mg of digestible lysine for laying hens. Lysine significantly

influences body (Valerio *et al*., 2003) and egg protein accretion (Ribeiro *et al.*, 2002), and therefore its intake is highly important for body growth and egg production.

Lysine is considered the most important dietary amino acid required for protein accretion for meat (Han and Baker, 1991) and egg production in poultry (Lemme, 2010). Faria *et al.* (2003) achieved maximum egg production at 0.68% of total dietary lysine, while maximum egg weight and egg content measurements were attained at a level of 0.72%. It is more relevant to estimate daily intake of lysine by the hens rather than dietary concentration. Bregendahl *et al*. (2008) found that a total daily intake of 538 mg of true digestible lysine was the requirement for maximum egg mass production, while Schutte and Smink (1998) recommended 720 mg/h/d of faecal digestible lysine for a daily egg mass of 57 g and for the best feed efficiency. Bonekamp *et al.* (2010) investigated the true faecal digestible (TFD) lysine requirement for heavy and light strains of Lohmann hens from 24 to 60 wk of age. The hens were restrictively fed DBP with 550 to 800 mg of TFD lysine per hen per day. In light hens, the laying percentage plateaued at a daily intake of 600 mg of TFD lysine, and for heavy hens the laying percentage increased from 550 to 700 mg of TFD lysine intake, but no further improvement was noticed from 700 to 800 mg. Egg production and egg mass were increased, and feed conversion ratio was decreased with TFD lysine BP intake of 800 mg/h/d in both the strains.

2.3.3. Threonine

Threonine was discovered independently as the last EAA by Maeda (1933) and Rose and Meyer (1936). Threonine is often the third limiting amino acid in commercial poultry diets. This EAA is vital for deposition of proteins in the body, and is mainly found in tissues of heart, nervous system and muscles. Threonine is also abundantly present in the mucus on the lining of gastrointestinal tract, which acts as a barrier to protect the bird from pathogens. There are few

published estimates of the laying hen's requirement for threonine. The NRC (1994) recommends a daily intake of 470 mg of total threonine for laying hens. Faria *et al.* (2002) obtained a maximum egg production response when diets contained 500 mg/100 g of diet of total threonine. Figueiredo *et al.* (2012) suggested 620 mg/h/d as the total threonine requirement. Threonine is available in the market in crystalline form and is commonly used for balancing protein in laying hen diets.

2.3.4. Tryptophan

In addition to lysine and total sulfur amino acids, many poultry nutritionists formulate diets with a minimum dietary tryptophan level. It is fourth limiting AA in commonly used corn and soybean meal based poultry diets after methionine, lysine and threonine. Older studies suggest a daily total tryptophan intake requirement of 100 to 200 mg (Jensen *et al.,* 1990) for laying hens, including a 160 mg of daily tryptophan recommendation by the NRC (1994). Lohmann Breeder Company has recommended a daily intake of 150 mg/hen of digestible tryptophan for LSL-Lite hens for a maximum egg output (Lohmann Management Guide, 2013). Peganova *et al.* (2003) reported that daily tryptophan requirement is somewhat dependent of dietary level of large neutral amino acids (LNAA) such as isoleucine, valine, leucine, phenylalanine and tyrosine. The authors suggested that at normal (adequate) levels of LNAA the tryptophan daily requirement for egg mass was 198 mg and the requirement was decreased to 155 mg/h/d when the LNAA level was increased by 40%.

2.3.5. Isoleucine, Leucine and Valine

Consideration of branched-chain AA (BCAA, isoleucine, leucine and valine), especially in low protein diets, becomes more important to ensure maximum performance. As more layer nutritional programs utilize synthetic threonine, it becomes necessary to understand isoleucine

dietary requirements. Not much research has been completed on the isoleucine requirement of laying hens. The NRC (1994) recommends 650 mg intake of total dietary isoleucine per day while Lohmann Breeder Company (Lohmann-LSL Management Guide, 2013) suggests 570 mg of digestible isoleucine at peak egg production (Lohmann Management Guide, 2013). A more recent study has shown that the margin between requirement and excess of isoleucine is very narrow in laying hens (Peganova and Eder, 2003). Increasing the diet total isoleucine concentration to 800 mg/kg of diet reduced body weight, and at 1000 mg/kg of diet, feed intake and egg performance were reduced as well. In the case of branched-chain amino acids dose-response studies, antagonisms should be considered as well. Isoleucine, leucine and valine not only share the common transport system through cell membranes, but they are also degraded by the same enzyme.

From the literature reviewed, there was no individual dose response study for leucine in laying hens. The level recommended by the NRC (1994) of 820 mg total leucine/h/d was estimated using a model.

There are only few dose response studies available for valine in laying hens. The NRC (1994) has recommended a total daily intake of 700 mg of valine, while Bregendahl *et al*. (2008) has suggested 501 mg of digestible lysine for a maximum egg mass response. Harms and Russell (2001) reported a maximum egg production and egg weight response at total valine intake of 592.5 and 677.7 mg/h/d, respectively. Peganova and Eder (2002) conducted three experiments using a dose response design for valine and found that a total of 609, 782 and 651 mg/h/d of daily valine intake was required for a maximum egg mass response of 50 g (24 – 32 wk of age), 58 g (25 – 32 wk of age) and 55 g (46 – 52 wk of age) respectively. These authors also reported

that a maximum nitrogen retention in body and egg was achieved at 594 mg of total valine intake per hen per day.

2.3.6. Arginine

Arginine is also an α – amino acid similar to lysine. In ideal protein diets, arginine becomes a limiting AA when lysine level is increased in the diet. Thus a higher level of arginine is needed with increased level of dietary lysine, and also because of an antagonistic relationship between lysine and arginine (Chapter 2.2.4). Feed ingredients like wheat and legumes are rich sources of arginine. The NRC (1994) has recommended a total daily arginine intake of 750 mg/h/d for maximum egg performance. Carvalho *et al.* (2012) has reported a maximum nitrogen intake at 862 mg/h/d of arginine with 700 mg/h/d of Dlys in laying hens.

2.4. Ideal amino acid or balanced protein concept

Conventionally, a minimum crude protein was set in a least-cost formulation system by using different feed protein sources and then AA were balanced by using supplemental AA. Mitchell (1964) suggested the concept of ideal protein or balanced protein (BP) for the first time. The BP concept was commercially used for the first time in Britain (ARC, 1981) by formulating swine diets based on balanced AA without considering a minimum CP level. This concept of diet formulation was called as 'ideal amino acid or an ideal protein concept'. This concept attains optimum animal performance based on an ideal proportion of dietary EAA. Thus, a diet based on an 'ideal protein or balanced protein concept' meets all the EAA requirements of birds for a desired performance response. Baker (University of Illinois) further studied this 'ideal protein concept' and formulated diets by incorporating all the EAA levels relative to the dietary lysine level (Baker and Han, 1994). Currently, many EAA are commercially available in the market in synthetic or crystalline form and they are considered as 100% digestible. With the availability of

crystalline AA, it has become much easier to formulate diets following ideal AA or balanced protein concept even at low CP levels (Burley *et al.,* 2013). In laying hens, the intake level of balanced AA should be adequate to maintain high level of egg production (Bregendahl *et al.,* 2008). The required levels of AA in the diet can be influenced by many factors such as level of egg production, egg weight or egg size, feed intake and barn management. Accordingly, dietary AA levels can be adjusted to ensure appropriate AA intake. If the level of balanced protein is too low at peak EP, it will result in reduced production of eggs and/or the production of smaller eggs. On the contrary, if the AA levels are higher than the hen's requirement, heavier eggs will be produced and that might lead to poor shell quality. There is an economic implication in both of the above situations. Thus, the nutritionist has to supply a balanced amount of digestible EAA and also in a timely manner. In broilers, the AA requirement is age dependent, however, the relative ratio of each EAA to lysine does not change.

In the ideal protein concept of diet formulation, lysine is considered the reference amino acid and all other amino acid levels are adjusted as relative to lysine level. Lysine is a leading AA in the concept BP as it has been considered most influential EAA for accretion of proteins in animals for meat, eggs and milk production. Also, more studies have been conducted on lysine and comparatively have more knowledge about its requirements and bioavailability in feed ingredients.

Hens source their amino acids from dietary protein and supplemental amino acids. Diets with ideal protein provide a balanced and adequate supply of EAA for attaining best egg and feed performance. Not many studies have been conducted to estimate the ideal AA requirements of laying hens. Some of the previous work in this regard have been shown in Table 2.1 where lysine has been considered as a reference amino acid for other EAA levels.

Table 2.1 Ideal amino acid recommendations for laying hens with lysine used as 100% of the requirement and other amino acids shown relative to lysine (%)

AMINO ACID	NRC[1] (1994)	CVB[2] (1996)	Jais *et al.* (1995)	Coon & Zhang (1999)	Bregendahl *et al.* (2008)	Lemme (2009)
Lysine	100	100	100	100	100	100
Arginine	101	-	82	130	-	104
Isoleucine	94	79	76	86	79	80
Methionine	43	50	44	49	47	50
TSAA[3]	84	93	-	81	94	91
Threonine	68	66	76	73	77	70
Tryptophan	23	19	16	20	22	21
Valine	101	86	64	102	93	88

[1]National Research Council; [2]Centraal Veevoederbureau; [3]TSAA: Total sulfur amino acids.

2.5. Response criteria considered for ideal AA research

The amino acid requirements of hens are dependent on the response criteria targeted such as egg production, egg size, and feed efficiency. Research on the AA requirements in hens has mostly targeted on egg production and feed efficiency variables, which are more associated with the economics of egg production. However, other characteristics of laying hens, which may not be of as much economic importance, are also of interest. For example, hen weight, body composition and feathering relate to hen obesity and welfare, and nitrogen excretion is linked to N and ammonia pollution of the environment.

2.5.1. Egg production

Egg production is an economically important criteria of a commercial egg operation. Therefore, it is important to understand the ideal AA requirements of laying hens for this trait. Older studies considered dietary protein as a requirement for maximum egg production (Pesti, 1991; Leeson and Caston, 1996), while more recent studies have estimated total or digestible AA requirements (Novak *et al*, 2006; Wu *et al*., 2007; Lemme, 2009). Today, poultry nutritionists use digestible AA in their diet formulations, often also incorporate ideal AA balance into feed formulation for laying hens. In an ideal protein concept, lysine is used as a reference AA and scientists have studied the digestible lysine requirement for maximum egg production. Some recent lysine intake recommendations for laying hens are shown in Table 2.2.

Table 2.2 Estimated lysine requirements for egg production in laying hens

Authors	Year	Lysine (mg/hen/day)	Total or digestible	Regression method
Schutte and Smink	1998	540	Apparent faecal digestible	Non-linear
Bregendahl *et al.*	2008	482	True digestible	Broken-line
Bonekamp *et al.*	2010	600	True faecal digestible	Linear and exponential
Filho *et al.*	2010	684	Digestible	Polynomial
Van Krimpen *et al.*	2015	810	Apparent faecal digestible	Meta-analysis

2.5.2. Egg weight and egg mass

Egg weight and egg mass are important parameters of laying hen performance and are influenced by the AA intake of laying hens (Samie and Pur, 2007). Commercial egg size classifications (extra-large, large, medium, small, etc.) are based on egg weight and the

distribution of egg classifications strongly impacts the economics of egg production. In some markets where eggs are used not used for shell egg sales (breaker market), output of egg mass (combines egg production and egg weight) is of greater economic significance. In addition, egg mass is a more accurate indicator of nutrient output from hens than either egg production or egg weight individually.

NRC (1994) did not list AA requirements specifically for egg weight or egg mass, but more recent work has used the ideal AA balance approach to study the hen's requirement for these parameters. Bregendahl *et al.* (2008) reported that 649 and 538 mg/h/d of true digestible lysine was required for a maximum response for egg weight and egg mass, respectively. A much higher requirement for digestible lysine (800 mg/h/d true faecal digestible lysine) was reported by Bonekamp *et al*. (2010) for a maximum response both egg weight and egg mass. A more recent meta-analysis study by Van Krimpen *et al.* (2015) has also revealed a similar report of 810 mg/h/d/ of faecal digestible lysine for maximum egg mass.

2.5.3. Feed intake and feed efficiency

Feed provides all the essential nutrients required for egg production, thus its intake level is vital for egg performance. In turn, feed intake also is important in establishing the levels of essential nutrients required to meet the hen's daily requirements. Of major economic consequence in egg production is the efficiency of feed utilization. Feed intake and feed efficiency are influenced by the dietary level of balanced AA (Shim *et al.*, 2013). An ideal protein diet should be adequately balanced for energy (ME) level to allow maximum utilization of dietary protein and amino acids. Studies have shown that increasing dietary lysine level impacts feed intake (Prochaska *et al.,* 1996, quadratically in experiment 2) and improved feed efficiency (Nathanael and Sell, 1980, experiment 1). Rocha *et al.* (2009) reported a linear

increase in lysine and feed intake, and a decrease in feed conversion ratio in a similar fashion when the dietary digestible lysine level was gradually increased from 0.545 to 0.770% from 24 to 40 wk of age. Novak *et al.* (2004) did not see any effect on feed intake and feed conversion ratio when two groups of hens consumed lysine 860 and 959 mg/h/d respectively. In that study, possibly, the gap between the low and high lysine intake was not big enough to produce the effect. Feed intake and feed efficiency have separate requirements for lysine in a balanced protein diet but most lysine estimation studies have considered only feed efficiency as their criteria for estimation. Bregendahl *et al*. (2008) has suggested 693 mg/h/d of true digestible lysine (broken-line regression method) for the best feed utilization by the hens. However, a more recent meta-analysis study by Van Krimpen *et al.* (2015) has reported a much higher lysine (apparent faecal digestible) requirement of 825 mg/h/d for a minimum feed conversion ratio. Feed intake and feed efficiency may directly impact the overall return on investment, therefore, these two criteria should also receive major attention in AA estimation studies of laying hens. In summary, a balanced approach is applied when formulating commercial laying hen diets and should contain adequate levels of ME and BP to achieve the desired FI and FCR levels along with a maximum egg response.

2.5.4. Hen weight and body composition

Protein and amino acid intake can influence hen weight gain with the response dependent on both level and balance of AA. When intake of balanced protein or AA is lower than the hen's requirement, hens gain less or in more severe deficiencies actually lose weight (Shim *et al*., 2013).

Bonekamp *et al.* (2013) reported a non-linear increase in hen weight in Lohmann Brown Classic (heavy breed) and Lohmann LSL Classic (light breed) when AA-balanced true fecal

digestible lysine intake of hens was gradually increased from 550 to 800 mg/h/d from 24 to 60 wk of age. In that study, hens consuming the lowest amount of 550 mg of AA-balanced true fecal digestible lysine reduced in weight in both the strains. Hens gained more weight when they consumed diets with higher levels of balanced protein in four phases between 19 to 74 wk of age with high (21.62, 19.05, 16.32, and 16.05% CP), medium (2.0% lower than high) and low (4.0% lower than high) balanced protein diets (Shim *et al*., 2013). In another study by Novak *et al*. (2004), hens gained more weight when consumed 959 mg/h/d of dietary lysine compared to 860 mg/h/d in phase II of the experiment. When BP is consumed above the requirement, more AA are deaminated and the carbohydrate fraction is turned into body fat (Campbell *et al*., 1984).

2.5.5. Plumage condition

Most of the body of adult birds is covered with feathers, except for the beak, lower legs and feet. Mature feathers do not contain blood vessels or nerves (Lillie, 1948). Structurally, feathers are approximately 90% protein, mainly beta-keratin. The amount of feathers is mostly dependent on the body mass of the bird and it usually accounts for an average 6% (4 - 8%) of the body mass (Stettenheim, 2000). Like other birds, laying hens use their feathers to perform many functions such as flight, body protection from external factors, water repulsion, and thermo-insulation and body temperature maintenance. Nichelman *et al.* (1986) reported an increase in heat production to compensate for the increased heat loss in poorly feathered hens. Heat loss is higher in hens with poor plumage condition and as a consequence more dietary energy is directed to maintenance at low barn temperatures; in turn hens increase feed intake to provide the extra energy maintenance requirements (Glatz, 2001). On the other hand, feathers also prevent heat loss when birds are exposed to higher environmental temperatures, and this can increase heat stress. Peguri and Coon (1993) conducted an experiment to study the impact of level of feather

cover (FC; 0, 50 and 100%) at three room temperatures (12.8, 23.9 and 33.9°C) on egg performance and nutrient intake. Feed intake of all the FC hens housed at 23.9°C was 115 g/b/d and it was 25 g/bird higher than FI of hens housed at 33.9°C and 13 g/hen lower than hens housed at 12.8°C. The hen-day egg production for hens at 12.8°C and 33.9°C was lower by 7.5 and 6.7%, respectively, compared to hens at 23.9°C. The metabolizable energy requirement for hens with 0% FC at 12.8°C was twice that of similar FC hens at 33.9°C. The results of this study indicate that feather condition can easily impact the economic returns of egg production by affecting feed intake and other egg performance criteria.

Cannibalism due to feather pecking is a common vice in laying hens. Studies have reported that bird genetics, environmental and nutritional influences are main factors which may trigger feather pecking in birds (Savory, 1995). Reports have also shown that the dietary deficiency of protein (Ambrosen and Petersen, 1997), and amino acids such as lysine (Al Bustani and Elwinger, 1987) and methionine plus cysteine (Elwinger, 2002) result in poor feathering. Broiler chicken feather keratin is rich in arginine, leucine, valine and cysteine AA but low in lysine and methionine (Stilborn *et al*., 1995). Methionine is certainly required for body growth, but its direct role in improving plumage condition is not clear. Danner and Bessei (2000) have reported a reduction in cannibalism and improvement in feathering when dietary methionine was increased from 0.22 to 0.36% in White Leghorn hens. Probably, methionine supplementation increased the total sulfur amino acid level in the diet helping the feathers to improve. Chickens fed poorly balanced diets with inadequate protein tend to pull and eat feathers of other birds in order to meet their protein requirement. In a study by Ambrosen and Petersen (1997), feather score was improved when dietary CP was increased from 11.1 to 19.3%. Thus, as a nutritional effect,

feather pecking and cannibalism are the reflection of behavioural responses of hens towards protein deficiency in their diet.

2.5.6. Egg component measurements

A normal table egg mostly consists of 9-12% egg shell, 30-33% yolk and approximately 60% albumen of total egg weight (Stadelman and Cotterill, 1995). Both external (egg shell) and the internal (albumen and yolk) qualities determine the overall quality of an egg. With ever increasing consumer concern regarding food quality, it is important that shell eggs meet consumer quality demands. Absolute egg component measurements change with egg size. Since egg weight is primarily influenced by the amount of amino acids consumed by the hens, similarly the absolute egg component measurements will also be affected in a similar fashion (Prochaska *et al*., 1996). Balanced protein provides necessary AA for egg protein synthesis, but the level of protein accretion is dependent on the hen genetics. Most studies have shown that egg component measurements as a proportion of egg weight do not change with increase in egg weight (Rocha *et al.,* 2009; Filho *et al.,* 2010). Studies also show that individual amino acids, especially lysine (Gunawardana *et al*., 2008), lysine and methionine (Novak *et al.*, 2004), intake levels significantly influenced the egg component measurements by impacting the egg weight. This is possible because lysine is the key AA required for egg protein synthesis.

2.5.6.1. Egg shell quality

Egg shell quality (cracks and other deformities) is an important aspect of overall egg quality. Egg shell quality can be analysed directly by measuring shell breaking strength, and also by indirect methods such as egg specific gravity (ESG), egg shell thickness or egg shell weight (Roberts, 2004). Dietary nutrients like protein and amino acid intake levels can easily influence egg size and it is possible that the egg shell quality may be affected by an increase in egg size. It

has been suggested that as egg size increases, egg shell does not increase proportionally. This is supported by decreasing ESG with increasing EW (Liu *et al*., 2005; Wu *et al.*, 2005). Studies also show that the egg shell quality declines with increasing age of the laying hens, which corresponds to increasing egg size (Roland, 1979; Roberts and Ball, 2004). The nature of the decline in egg shell quality with increasing egg size is strain dependent as shell quality varies with strain of laying hens (Curtis *et al*., 1985; Gunawardana *et al*., 2008).

2.5.6.2. Internal egg quality

Internal egg components, albumen and yolk, are equally important from egg quality and egg economics perspectives. Quality of albumen and yolk is greatly influenced by handling, and storage length and conditions. The rate of change in albumen and yolk quality is largely dependent on the temperature and humidity of the egg storage room, and also on the rate of loss of carbon dioxide (CO_2) gas through the egg shell. Hen nutrition also plays an important role in producing eggs with good quality of albumen and yolk.

Internal egg quality of albumen can be monitored by using different methods such as measuring the albumen height (AH) or calculating Haugh units. Haugh units have traditionally been used to determine the level of egg protein and freshness, and values are dependent on height of thick albumen and egg weight. However, research has suggested no clear advantage of Haugh units over albumen height. Silversides (1994) compared the techniques and suggested that albumen height measurement is more valid to measure albumen quality because of a poor statistical correlation between an EW and AH. He also reported that the Haugh unit is more dependent on AH than albumen or egg weight, except for eggs stored for an extended period of time (three weeks). As noted above, an increase or decrease in EW affects the absolute weights of albumen and yolk, and possibly can also affect proportional measurements. Thus, nutritional

factors such as energy, protein and amino acids can dictate internal egg component measurements by impacting the EW (Gunawardana *et al*., 2008). Research on the impact of dietary protein and amino acids on albumen quality has not been conclusive. Results have varied from an increase in albumen height or Haugh units with varying protein or AA intake levels (Hammershoj and Kjaer, 1999; Balnave *et al*., 2000). No explanation is obvious for the variation in response and a decrease in albumen height with increasing egg size is counter-intuitive based on the absolute amount of albumen increasing with egg size. A possibility is that the proportion of thin and thick albumen changes with quality of protein accretion in albumen when egg size increases, especially for ovomucin albumen protein. Level and quality of ovomucin protein in albumen influences thick albumen viscosity and ultimately the albumen height. More thin albumen inclusion in larger eggs could have an impact on the height of the remaining albumen. Intake level of balanced protein by the hens also impacts internal egg component measurements.

Yolk is synthesized by the liver and is continuously deposited in the ovum. Albumen is added later in the magnum (part of the oviduct) for providing microbial and mechanical protection to the yolk. The pH of yolk in a freshly laid egg is 6.0 and it gradually rises with age of the egg (Stadelman and Cotterill, 1994). Nutrition of hens can influence the yolk and albumen measurements. Shim *et al*. (2013) reported an increase in absolute YW and a decrease in albumen Haugh units with increase in egg size when four levels of dietary protein were provided to hens in a phased feeding program from 23 to 74 wk of age. However, the yolk weight as proportion of EW was not affected in that study. Wu *et al.* (2007) has also reported that a dietary increase in protein level increased yolk and albumen absolute weights, however, the treatment effect was more significant on absolute AW than absolute YW. That means, with increasing protein intake and EW, albumen to yolk ratio decreases. Balanced protein intake levels also

influence internal egg composition. Prochaska *et al.* (1996) reported a positive quadratic response for absolute AW and YW when lysine intake increased from 638 to 1165 mg/h/d. Gunawardana *et al.* (2008) also found significant effect on yolk and albumen proportion weights when hens consumed three levels of dietary lysine (849, 924 and 1023 mg/h/d) from 21 to 36 wk of age.

2.5.7. Nitrogen excretion

Animal production has been blamed for polluting the environment with nitrogenous compounds (nitrogen, ammonium, ammonia) and green-house gases (nitrous oxide). Animal agriculture contributes significantly to the total volatization of ammonia from organic nitrogenous compounds. Poultry species excrete high amounts of nitrogenous content in their excreta. Under intensive production systems, poultry are fed diets with high levels of crude protein and amino acids to achieve high production output. Chickens can only utilize approximately 40% of the total dietary protein they consume (Mousavi *et al*, 2013). Since hens do not have an efficient protein storage mechanism, the unutilized protein and AA are deaminated in the liver into N and then excreted as urea (5%), uric acid (80%) and ammonium (NH_4, 10%) (Goldstein and Skadhauge, 2000). Under ideal environmental conditions of temperature and humidity, these organic N compounds (urea and uric acid) can be easily fermented by the microbes into NH_4, water and ammonia (NH_3) gas. Nitrogen and NH_4 are water soluble and can easily pollute soil and underground water sources. Ammonia is the major product of microbial fermentation and is emitted into the environment causing air pollution inside and outside of the poultry barn. Ammonia gas pollution does not cause global warming per se, but ammonia can be oxidized to nitrous oxide (NO_2) which is a green-house gas. Ammonia can also cause serious health hazards to the people working in the poultry barns and to

the birds as well. High barn ammonia level compromises poultry welfare (Dawkins *et al.*, 2004). Poultry nutritionists have developed several dietary strategies to minimize the risk of environmental pollution with nitrogenous compounds as a result of poultry production.

Several experiments have been conducted to study the link between dietary CP levels and excretion of nitrogenous compounds. Studies have showed that the total N excretion can be greatly reduced just by reducing the crude protein level in the poultry diets (Bregendahl *et al*., 2002). Blair *et al.*, (1999) has also reported significant reduction in excreta N contents without compromising the performance in broilers and layers by reducing the dietary CP with supplementation of crystalline AA. In a study by Latshaw and Zhao (2011), the manure N content ranged between 3.98 to 5.68% of manure DM when dietary protein intake was increased from 13 to 17 g/h/d. Excess consumption of dietary protein provides more unutilized NEAA available for deamination. However, a balanced protein diet is based on the birds' ideal AA requirements and less AA are available for deamination. Thus, it can be summarized that the level of N excretion in poultry can be reduced by formulating diets to meet the bird's EAA requirements and to contain sufficient dietary protein to provide N for synthesis of NEAA. To achieve this, first we need to know the ideal AA requirements of genetically changing laying hens.

2.6. Low protein diets for laying hens

Birds use dietary protein as a source of N to synthesize NEAA. If there is not enough nitrogen available in the diet, then EAA can be utilized to derive N required for synthesis of NEAA. This situation is uneconomical and can harm the performance of birds because of the partitioning of EAA for production to NEAA synthesis. Conventionally, dietary crude protein levels were used to formulate hen diets to meet the EAA requirements of hens resulting in higher

crude protein levels in the diets. Such diets provided nitrogen in excess of the requirement for NEAA synthesis. These diets can cost more and also result in increased N excretion by the hens.

Efforts have been made in the past to reduce the crude protein in the diets (Liu *et al*, 2005; Burley *et al*., 2013). This can only be possible when diets contain adequate amounts and ratios of all the EAA, and also there is also enough N available to synthesize NEAA. With the market availability of most crystalline EAA, it is possible to replace some of the intact dietary protein with synthetic EAA. In a study by Blair *et al*. (1999), the layer performance (egg production and proportion of egg components) was not affected when the dietary protein was reduced from 17.0% to 13.5% and AA-balanced lysine level was adjusted to 90, 100 and 110% of NRC recommendations by using crystalline amino acids. In another study, Latshaw and Zhao (2011) did not see any effect on egg and feed performance, and body weight when laying hens consumed 13, 15 and 17 g of crude protein per hen per day with a daily intake of AA balanced Dlys of 710 mg/h/d.

Studies also show that the low protein diets supplemented with EAA can produce good results, but not a maximum response. Keshavarz and Austic (2004) reported that the low protein diets can sustain good performance for a short duration only. They also mentioned that the digestibility and bio-availability of AA in feed ingredients and the improper ratio between the supplemental EAA in low protein diets may have been the reason for the inferior performance of laying hens. Probably, we need to understand the role of NEAA deeper in maximizing the performance of laying hens for successful incorporation of low protein in poultry diets.

The review of the literature has shed some light on how the laying hens' performance is impacted with different intake levels of protein and amino acids. It is also demonstrated that protein and amino acid levels affect some of the other important characteristics of egg

production, which may not be directly affecting the egg economics, but are certainly important from birds' welfare or environmental points-of-view. From this literature review, it can be hypothesized that the genetics changes in hen performance have affected the hen's requirement for balanced protein. Thus, the objective of this study was to assess the response of laying hens to graded intake levels of amino acid-balanced digestible lysine on egg and feed performance, and on body composition, feathering, egg quality and nitrogen excretion.

3. RESPONSE OF LAYING HENS TO GRADED LEVELS OF DIGESTIBLE BALANCED PROTEIN INTAKE ON EGG PRODUCTION, EGG WEIGHT, EGG MASS, FEED INTAKE, FEED EFFICIENCY AND EGG SIZE CLASSIFICATION

3.1. Abstract

Laying hens have increased in egg production and feed efficiency over last few decades. Thus, it is relevant to understand the amino acid (AA) requirements of hens to achieve their full genetic potential. Research using 600 Lohmann-LSL Lite hens compared graded levels of digestible AA balanced to Dlys on performance from 27 to 66 wk of age. There were five dietary treatments and hens were projected to consume 550, 625, 700, 775 or 850 mg/h/d of Dlys. Each treatment was replicated 10 times (2 conventional cages each housing 6 hens). Data collection included egg production (EP), egg weight, feed intake and the incidence of mortality. Based on these values, egg mass (EM), egg size classifications, feed efficiency (FE, kg feed / kg EM) and lysine efficiency (LE, mg Dlys / g EM) were calculated. The experiment was a completely randomized design and regression analysis was used to determine the nature of the response to dietary treatments. Differences were considered significant when $P \leq 0.05$. Hen-day (HD) and hen-housed EP, EW, EM, FI and LE increased in a quadratic manner with increasing Dlys intake, while the proportion of cracked eggs increased linearly. A negative quadratic relationship was found between Dlys intake and FE and mortality. By using a non-linear regression model, maximum HDEP, EW and EM, and minimum FE were achieved at 769, 903, 836 and 839 mg/h/d of Dlys intake respectively. The proportion of extra-large and jumbo size eggs increased linearly while the large eggs increased, and medium and small eggs decreased quadratically with

increasing Dlys intake. In conclusion, the AA balanced Dlys requirement of laying hens varies with the response criteria being assessed.

Key words: layers, ideal protein, lysine optimization, egg performance, feed utilization

3.2. Introduction

Laying hens have undergone extensive genetic selection for decades, and as a consequence their egg performance characteristics and feed efficiency have continuously increased (Anderson *et al*., 2013). With these changes, it is possible that their nutrient requirements may have changed to match the increase in egg mass output. Therefore, it is relevant to examine the response of hens to dietary digestible amino acid (AA) levels, as they are key nutrients required to permit expression of the genetic potential of selected genotypes (Bregendahl *et al.,* 2008). Further, the increased cost of protein and awareness of nitrogen pollution from poultry production are additional reasons to clearly understand the ideal protein requirement of laying hens.

Considerable research has examined the amino acid requirements of laying hens. In addition to using less productive hens of the respective time periods, the nature of older research also limits its value for today's hens. Use of less essential AA in the trial design, total rather than digestible AA and a lack of ideal AA balance are the key limiting factors.

Earlier studies have shown that the egg performance and feed efficiency of laying hens improves with increasing balanced protein and lysine intakes. For example, egg weight and EM were increased and FE improved with an increase in dietary Dlys level (Karunajeeva *et al.,* 1987). Recommendations for lysine, including both total and digestible values have varied and gradually increased over the years. The National Research Council (NRC, 1994) recommended a daily total lysine intake of 690 mg/h/d in a balanced protein for laying hens, while Centraal Veevoeder Bureau (CVB, 1996) recommended 700 mg/h/d of Dlys. Schutte and Smink (1998) recommended 0.66% of digestible dietary lysine or 0.82% of total dietary lysine to achieve the best feed efficiency. Coon and Jhang (1999) suggested 676 mg of Dlys for laying hens. In a more recent study, Bregendahl *et al.* (2008) has reported a total daily intake of 720 mg of lysine was

necessary for a maximum EM response. These studies reflect that the continuously increasing amino acid requirements of laying hens in line with the changing in their egg performance characteristics.

With availability of synthetic AA, it has become possible to formulate AA balanced diets with less emphasis on dietary protein level. But to attain success in that direction, it is necessary to clearly understand the digestible ideal amino acid requirements of laying hens. The objective of this study was to assess the response of laying hens to increasing levels of DBP (Dlys intake of 550, 625, 700, 775 or 850 mg/hen/day) from 27 to 66 wk of age. In line with previous research, it was hypothesized that the amino acid-balanced Dlys requirement of laying hens will be dependent on the specific response criteria being assessed.

3.3. Materials and methods

The Animal Care Committee of the University of Saskatchewan approved this animal experimentation and the experiment was conducted following the guidelines of the Canadian Council of Animal Care (1993) as specified in the Guide of the Care and Use of Experimental Animals.

3.3.1. Birds and housing

The experiment was a completely randomized design and a total of 600 hens were equally divided into five dietary treatment groups and each group was projected to consume 550, 625, 700, 775 or 850 mg/h/d of Dlys. Treatments were replicated 10 times with 12 birds per replicate (two adjacent cages with six birds per cage). The experiment was conducted from 27 to 66 wk of age.

The experiment was conducted using Lohmann-LSL Lite hens. Hens were beak-trimmed at the hatchery and raised on litter floor pens at the University of Saskatchewan Poultry Centre. During pullet rearing, nutrition and lighting programs approximated Lohmann recommendations (Lohmann-LSL Lite Management Guide, 2013). At 17 wk of age, pullets were randomly assigned to conventional cages (Specht cages with dimensions of 60.96 cm long x 39.37 cm wide, and 40 cm height) at the same site. From cage housing until the start of the experiment (27 wk), the birds were fed a commercial laying hen diet that met or exceeded Lohmann recommendations (Lohmann-LSL Lite Nutrition Management Guide, 2013). Before and after the start of the experiment, feed and water were provided *ad libitum*. One Lubing nipple drinker per cage (shared) was available to provide fresh drinking water. The laying barn was equipped with an automated ventilation and heating system, and the barn minimum temperature was maintained at approximately 20 to 22°C for the experimental period. Light was provided by incandescent bulbs for 14 hours per day at a light intensity of 10 lux at feeder level. At the start of the experiment, the average HW was 1,640 g and the HDEP was at 97.0%. Hens were healthy and mortality rate was lower than the Lohmann-LSL standard before the start of the experiment.

3.3.2. Feeds and feeding

Two isocaloric (2,900 kcal ME/kg of feed) base diets were prepared in mash form containing 500 or 850 mg of Dlys per 100 g of diet (Table 3.1). All other essential AA were balanced relative to Dlys level (100%) by following the recommendation of Bregendahl *et al.* (2008) for methionine (47%), TSAA (94%), threonine (77%), tryptophan (22%), and isoleucine (79%). Valine was added using the Lemme (2009) recommendation of 88% of the Dlys level.

Table 3.1 Ingredient and chemical composition of base diets containing 500 and 850 mg digestible lysine per 100 g of diet

Ingredients (%)	500 mg Dlys/100 g diet	850 mg Dlys/100g diet
Wheat	84.74	63.75
Soybean meal	0.00	18.84
Canola oil	2.39	4.55
Limestone	10.40	10.29
Mono di-calcium phosphate	1.22	1.22
Layer vitamin-mineral premix[1]	0.50	0.50
Sodium chloride	0.33	0.35
DL-Methionine	0.06	0.27
L-Lysine HCL	0.19	0.01
L-Threonine	0.04	0.10
Endofeed W[2]	0.03	0.03
Calculated Composition (%)		
AME (kcal/kg of diet)	2,900	2,900
Crude protein	14.40	19.95
Crude fat	4.06	5.94
Calcium	4.10	4.10
Chloride	0.27	0.27
Non-phytate phosphorous	0.42	0.42
Total phosphorous	0.56	0.60
Potassium	0.39	0.67
Sodium	0.17	0.17
Linoleic Acid	1.38	1.63
D-Arginine	0.57	1.066
D-Isoleucine	0.41	0.67
D-Leucine	0.88	1.28
D-Lysine	0.50	0.85
D-Methionine	0.24	0.5
D-Methionine + cysteine	0.50	0.8
D-Threonine	0.39	0.65
D-Tryptophan	0.11	0.22
D-Valine	0.55	0.77

[1] Provided per kilogram diet: vitamin A, 8000 IU; vitamin D, 3000 IU; vitamin E, 25 IU; menadione, 1.5 mg; thiamine, 1.5 mg; riboflavin, 5 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.01 mg; niacin, 30 mg; pantothenic acid, 8 mg; biotin, 0.06 mg; copper, 10 mg; iron, 80 mg; manganese, 80 mg; iodine, 0.8 mg; zinc, 80 mg; selenium, 0.08 mg; and calcium carbonate, 500 mg.

[2] Feed enzyme supplied by GNC Bioferm (Box 6, Bradwell, Saskatchewan, S0K 0P0, Canada) containing a minimum 700 units per gram of beta-glucanase and a minimum 2,250 units per gram of xylanase.

The AA digestibility values and ME levels of the feed ingredients of the base diets were based on the amino acid database of Evonik Degussa (Evonik AMINOdat 4.0). The energy level (ME; 2,900 kcal/kg of diet) of the base diets was set slightly higher than recommended to ensure that it was not limiting in the experimental diets. Limited energy might result in the use of EAA to supply the energy requirements of the hen. The five treatment diets were manufactured by blending the two base diets in calculated proportions to provide the targeted intake levels of Dlys per bird per day. The average feed intake in the preceding three weeks period was used to calculate the mixing proportions of the base diets (Table 3.2).

Table 3.2 Relationship between hen feed intake and the level of digestible lysine (Dlys) in experimental diets

Treatment intake Dlys (mg/h/d)	Hen feed intake (g/h/d)				
	95	100	105	110	115
	Diet % Dlys				
850	0.895	0.850	0.810	0.773	0.739
775	0.816	0.775	0.738	0.705	0.674
700	0.737	0.700	0.667	0.636	0.609
625	0.658	0.625	0.595	0.568	0.543
550	0.579	0.550	0.524	0.500	0.478

3.3.3. Data collection

Eggs were collected manually five days a week and eggs numbers were then mathematically corrected to determine weekly percent HDEP and HHEP. Abnormal eggs such as misshaped, cracked, soft-shelled, double-yolks and broken were collected and counted separately, but were included in the total egg production values. The amount of feed consumed was measured every three wk during the experiment and then average FI (g/h/d) per replicate cage was calculated by

using following calculation: amount of feed consumed (g) / total number of hen-days per replicate. Egg weight and egg mass were also measured every three wk. All the eggs from one day were individually collected, counted, and weighed to the nearest 0.1 g. Average EW (g) was calculated by dividing the total EW by the total number of eggs for each replicate. Egg mass (g) was then calculated per replicate by using the following equation: laying rate x average EW (g) for each 3 week time period. Feed efficiency (FE) as assessed by feed: EM ratio (kg feed / kg EM) was calculated every three wk. Based on the recorded individual EW, all the eggs produced during this experiment were classified by size as jumbo (more than 70.0 g), extra-large (63.0-70.0 g), large (56.0-63.0 g), medium (49.1-56.0 g), small (42.0-49.0 g), and peewee (less than 42.0 g) by following the Canadian regulation of egg size classification (Egg Size Regulation, Schedule III of Sections 14 and 31, under Consolidated Regulations of Canada Laws). Dead and cull birds were collected as they occurred or were identified, and were sent to Prairie Diagnostic Laboratory at the University of Saskatchewan for necropsy to determine the cause of death. Total mortality for each treatment was calculated as a percentage of the number live birds housed at the start of the experiment.

3.3.4. Statistical analysis

The means of the responses were derived with one-way ANOVA of mixed model. The data were statistically analysed using PROC REG (regression) and PROC RSREG (response surface regression) of Statistical Analysis Systems 9.3 (SAS, 2003). The level of significance was fixed at $P \leq 0.05$. The levels of Dlys intake, as a balanced protein, required for maximum HDEP, EW, EM, and for minimum FE were calculated separately by using the following regression model:

$y = ax^2 + bx + c$; where y = parameter being assessed (dependent variable), x = Dlys level (independent variable) and c = intercept.

The minimum (x1) and maximum (x2) values of x variable were calculated as a quadratic function ($ax^2 + bx + c = 0$). The mean of the two x values was considered to be the Dlys intake level required to produce the maximum or minimum response for the criteria being assessed.

3.4. Results

Actual Dlys intakes were calculated using feed intake and the Dlys levels of the diet, and derived values were close to the targeted levels (Figure 3.1). The actual Dlys intakes were used in statistical evaluation of the response data.

Egg production (both HD and HH), egg weight, egg mass, feed intake and Dlys to egg mass ratio were increased, and feed to egg mass ratio decreased in a quadratic manner with increasing intake levels of Dlys (Table 3.3). Dietary treatment did not affect levels of abnormal eggs (double-yolks, misshaped, soft-shelled or broken eggs; data not shown). However, the proportion of eggs classified as cracked increased linearly with increasing Dlys intake. The incidence of mortality decreased (quadratic) with increasing Dlys intake. The mortality response can be primarily attributed to the high level of loss (11.7%) for the 560 Dlys intake treatment. The loss for the hens in the latter treatment was mainly due to body emaciation and cannibalism which accounted for 50.0% of the total mortality. The proportion of jumbo and extra-large eggs increased linearly with Dlys intake, while proportions of large, medium and small eggs ($P < 0.0001$) were affected in a quadratic manner (Table 3.4). There was no production of eggs weighing 42 g or less (peewee eggs) in this experiment.

Regression analyses were used to estimate the maximum or minimum response to Dlys intake as a balanced protein. The maximum response on HDEP (Figure 3.2), EW (Figure 3.3)

and EM (Figure 3.4), and the minimum response for FE (Figure 3.5) were estimated at 769, 903, 836 and 839 mg/h/d of Dlys intake, respectively.

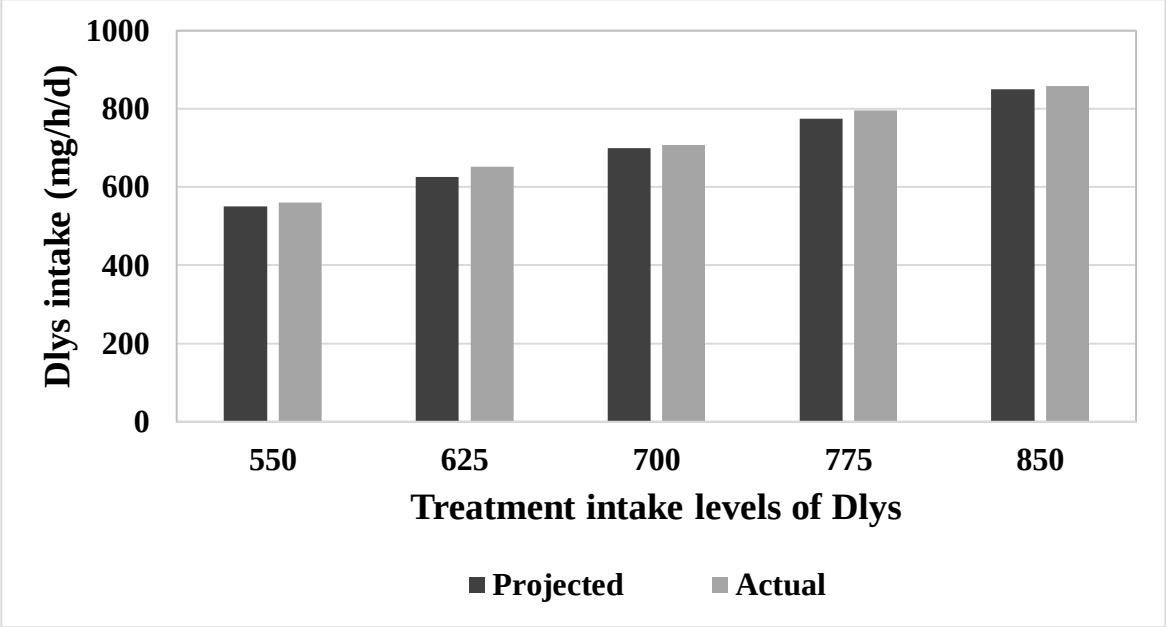


Figure 3.1 Digestible lysine intake during the experiment, projected vs. actual.

Table 3.3 Effects of digestible lysine (Dlys, balanced protein) intake levels on egg production, egg weight, egg mass, feed intake, feed efficiency and mortality of laying hens from 27 to 66 wk of age

	Dlys intake (mg/h/d)[1]						
Parameters[2]	560	651	707	795	858	SEM[3]	Regression equation[4]
HD egg production (%)	83.8[5]	90.9	93.1	93.6	93.3	0.72	$y = -0.000211x^2 + 0.3244x - 30.3923$
HH egg production (%)	80.0	89.8	92.7	92.6	92.0	0.92	$y = -0.000302x^2 + 0.458296x - 80.01396$
Cracked eggs (%)	0.08	0.04	0.10	0.12	0.11	0.01	$y = 0.0002x - 0.0473$
Egg weight (g)	55.4	58.4	59.4	61.0	61.7	0.11	$y = -0.00004977x^2 + 0.08996x + 21.1583$
Egg mass (g)	46.5	52.7	55.4	57.6	57.6	0.69	$y = -0.00016x^2 + 0.267125x - 53.4975$
Feed intake (g/h/d)	103.4	106.8	107.1	107.2	107.1	0.70	$y = -0.00009193x^2 + 0.139176x + 54.9950$
FE (kg feed/kg EM)	2.24	2.03	1.93	1.86	1.86	0.02	$y = 0.0000052x^2 - 0.00868x + 5.4894$
LE (mg Dlys/g EM)	13.4	13.1	13.2	14.2	15.5	0.02	$y = 0.000041991x^2 - 0.054179 + 30.5938$
Total mortality (%)	11.7	3.3	2.5	3.3	3.3	0.17	$y = 0.00023x^2 - 0.3480x + 131.7947$

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.

[2] HD, hen day; HH, hen housed; EM, egg mass; FE, feed efficiency; LE, digestible lysine efficiency.

[3] SEM: Pooled standard error of mean.

[4] Equations were considered significant if $P \leq 0.05$.

[5] The values are means from 10 replications consisting of two adjacent cages with six hens per cage.

Table 3.4 Effect of digestible lysine (Dlys, balanced protein) intake levels on egg sizes as a percent of total egg production in laying hens from 27 to 66 wk of age

	Dlys intake (mg/h/d)[1]						
Egg Sizes[2]	560	651	707	795	858	SEM[3]	Regression equation[4]
Jumbo	0.3[5]	0.2	0.9	1.9	2.6	0.14	$y = 0.0084x - 4.7963$
Extra large	4.6	10.8	17.0	27.1	33.7	0.69	$y = 0.0996x - 52.5307$
Large	39.0	59.7	61.6	58.5	55.9	0.72	$y = -0.00066x^2 + 0.9855x - 304.6634$
Medium	49.3	28.5	19.8	12.5	7.7	0.81	$y = 0.00039x^2 - 0.6928x + 313.7613$
Small	6.8	0.8	0.7	0.0	0.1	0.18	$y = 0.00015x^2 - 0.2338x + 90.2166$
Peewee	0.0	0.0	0.0	0.0	0.0	0.00	NS[6]

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.

[2] Jumbo, >70.0 g; extra-large, 63.0-70.0 g; large, 56.0-63.0 g; medium, 49.0–56.0 g; small, 42.0-49.0 g; peewee, less than 42.0 g.

[3] SEM: Pooled standard error of mean.

[4] Equations were considered significant if $P \leq 0.05$.

[5] The values are means from 10 replications consisting of two adjacent cages with six hens per cage.

[6] NS: Not significant.

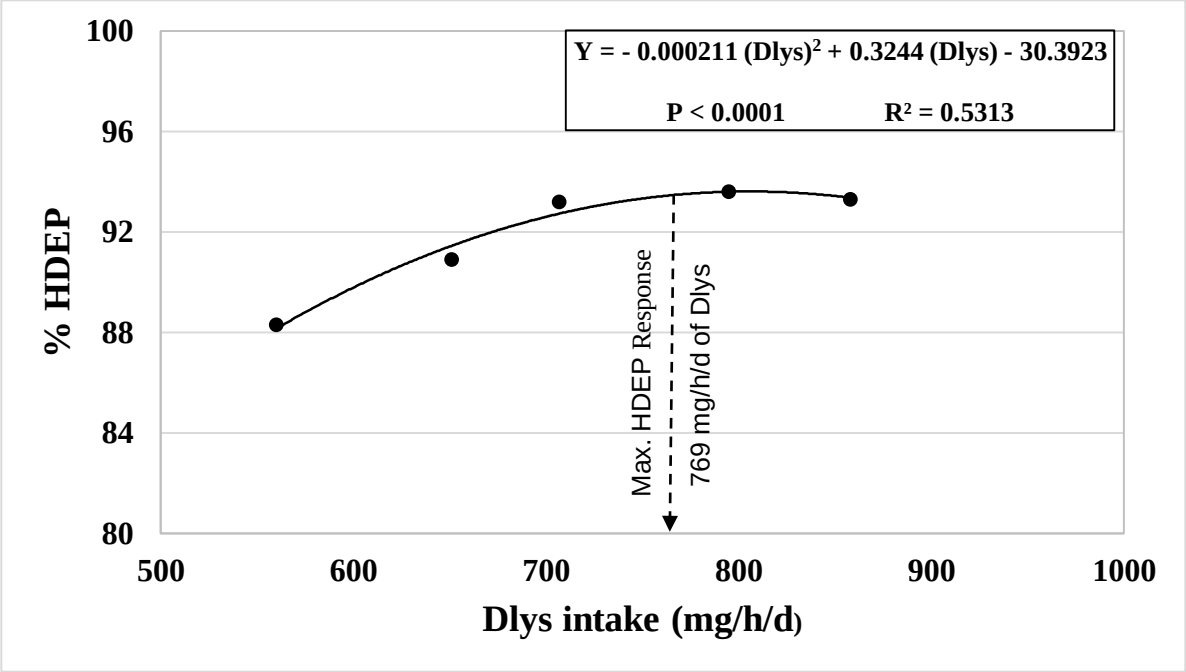
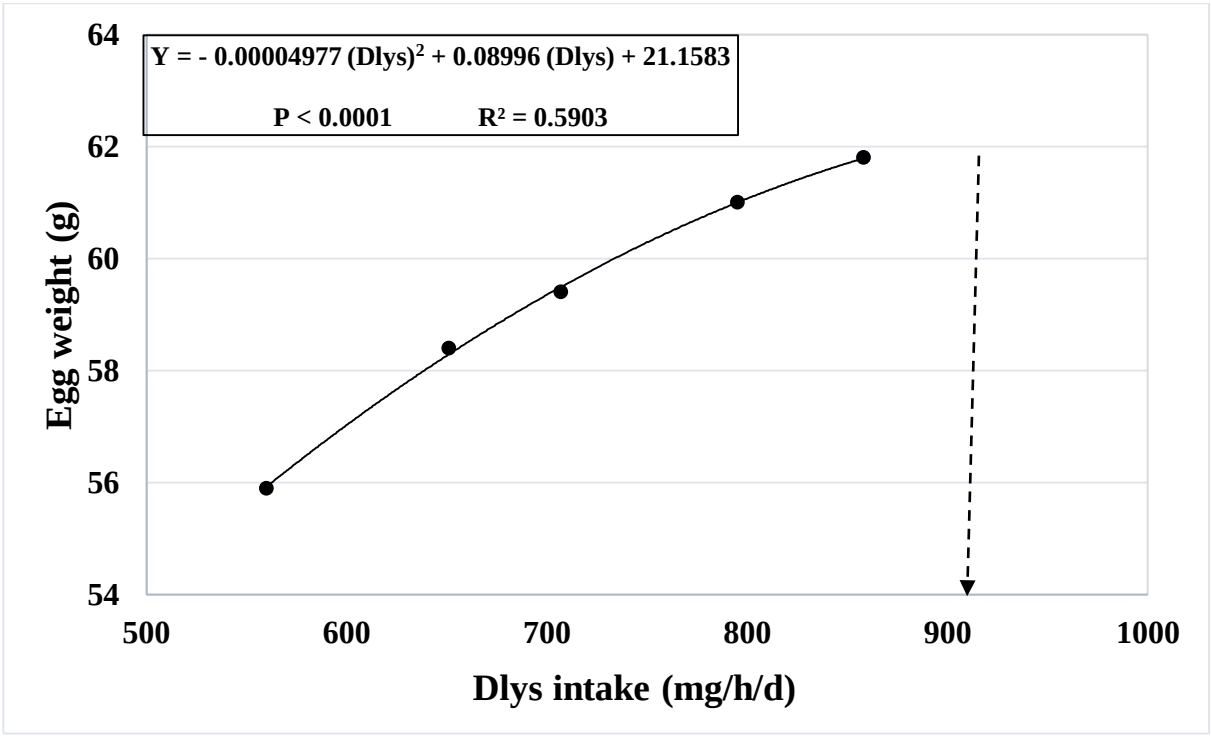


Figure 3.2 Effect of digestible lysine (Dlys) intake on hen-day egg production (HDEP) of laying hens from 27 to 66 wk of age.



.

Figure 3.3 Effect of digestible lysine (Dlys) intake on egg weight of laying hens from 27 to 66 wk of age.

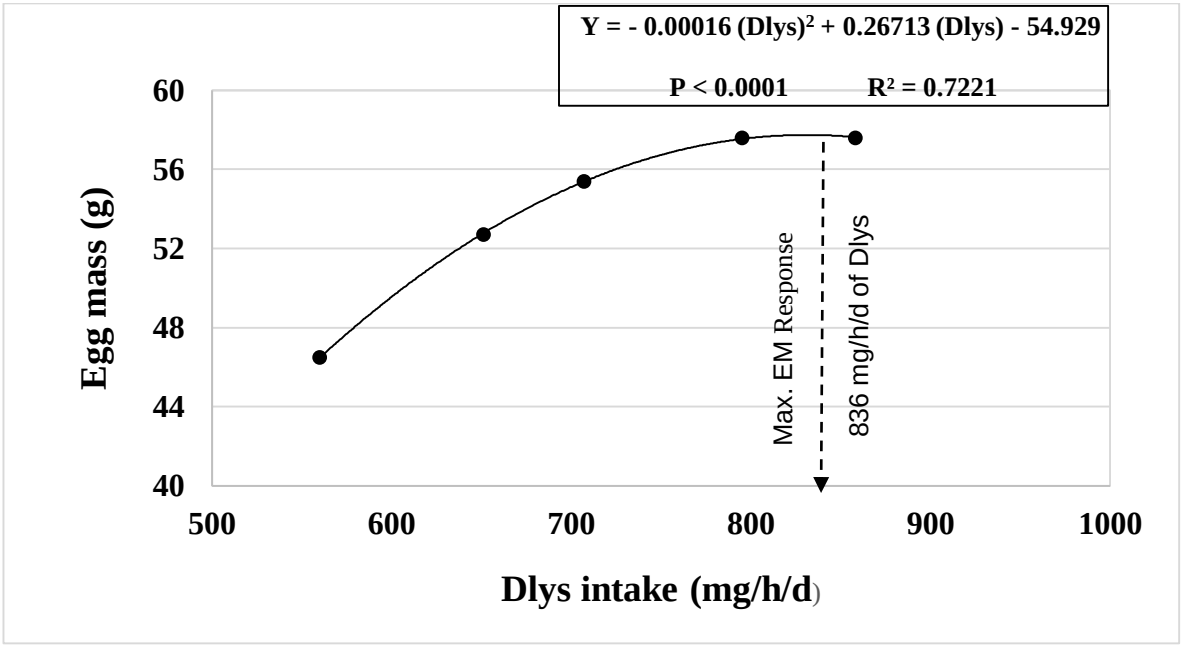


Figure 3.4 Effect of digestible lysine (Dlys) intake on egg mass (EM) output of laying hens from 27 to 66 wk of age.

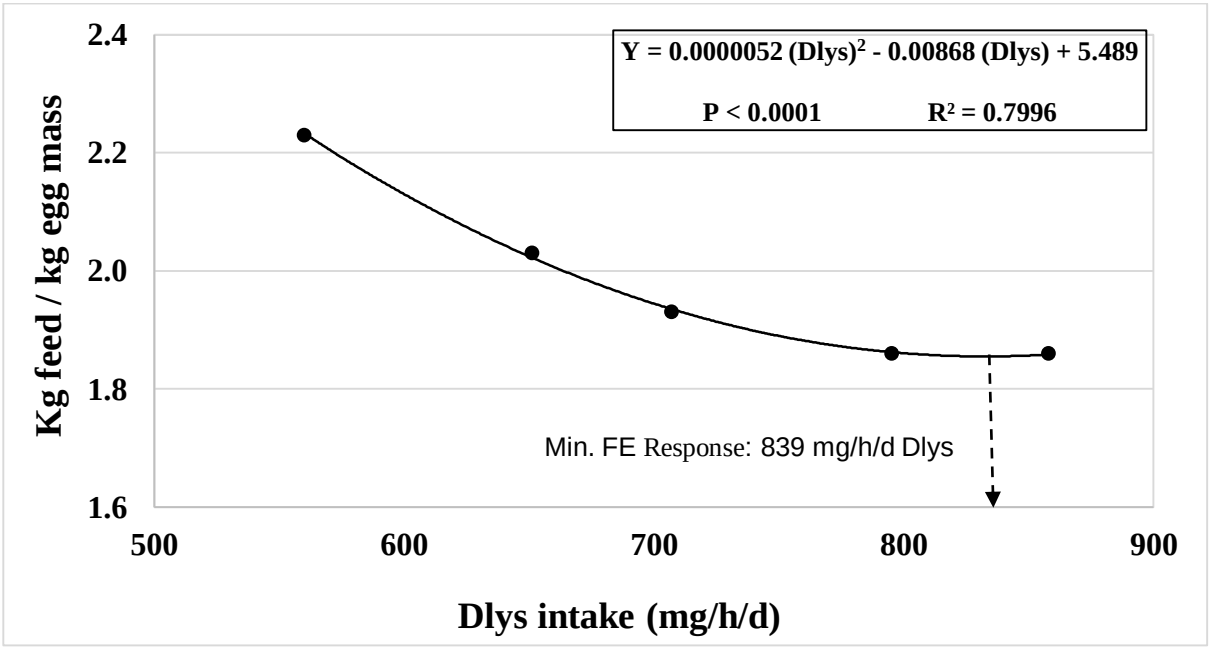


Figure 3.5 Effect of digestible lysine (Dlys) intake on feed efficiency (FE) of laying hens.

3.5. Discussion

3.5.1. Egg Production

Hen-day egg production in this experiment was comparable to those listed in the breeder's performance objectives (for all treatments except for the hens consuming BP with 560 and 651 mg of Dlys, which produced an average HDEP of 83.8 and 90.9%, respectively), compared to the breeder's recommendation of 93.7% of average HDEP from 27 to 66 wk of age (Lohmann-LSL Lite Management Guide, 2013). The total HDEP was significantly increased at 707 mg Dlys intake and a plateau for peak egg production was reached between 707 and 795 mg/h/d. In the current study, the HDEP increased with BP Dlys intake level in a quadratic fashion, which is in agreement with the response pattern observed by others (Nathanael and Sell, 1980; Silva *et al*., 2013). Bonekamp *et al*. (2010) also reported an increase in laying percentage of Lohmann-LSL Classic hens from 24 to 60 wk of age when TFD lysine intake was increased from 550 mg to 800 mg/h/d. In contrast, Schutte and Smink (1998) did not find any effect on HDEP when apparent fecal digestible lysine was increased from 539 to 847 mg/h/d from 24 to 36 wk of age. Similarly, Figueiredo *et al*. (2012) documented no significant effect of Dlys levels (0.675 to 0.875% of the diet) on egg production from 42 to 58 wk of age in Hy-Line layers. The increase in cracked eggs associated with increasing Dlys intake suggests a reduction in shell quality due to an increase in egg size (Table 3.4). This corresponds to a decrease in egg shell proportional weight (Chapter 4.6, Table 4.3) seen with increasing intake of Dlys.

3.5.2. Egg weight and egg mass

Overall EW was significantly affected by dietary treatment (Table 3.3). A peak response on EW was not observed in this experiment as the slope of the response curve continued to rise beyond the value for the highest Dlys intake. The statistical analysis of the data predicted a

strong quadratic relationship between Dlys intake and egg weight, and extrapolated to predict a maximum response (61.8 g) at 903 mg/h/d Dlys intake. A similar quadratic response was also reported by Nathanael and Sell (1980) and Cupertino *et al.* (2009). Figueiredo *et al.* (2012) reported a maximum EW response of 63.3 g at a Dlys intake of 798 mg/h/d from 42 to 58 wk of age, which is much lower than the Dlys level (903 mg/h/d) estimated in the current study. Keshavarz and Jackson (1992) and Novak *et al.* (2004) also documented an increase in EW when hens consumed diets with higher lysine levels. In the current study, the mean EW was the lowest (55.4 g) at 560 mg and the highest (61.7 g) at 858 mg of daily Dlys intake, which is close to the mean EW of 62.1 g for Lohmann-LSL Lite hens from 27 to 66 wk of age (Lohmann-LSL Lite Management Guide, 2013). However, the highest absolute EW achieved in the last three weeks of this study was 56.6, 60.1, 61.0, 63.6 and 64.9 g at five increasing intake levels of Dlys. The EW curve did not reach a plateau (Figure 3.3) in this experiment and a higher Dlys intake was required to attain the predicted peak EW response.

Because of the strong quadratic response of Dlys on HDEP and EW, the response for EM was also similar. This is in close agreement with the findings of Silva *et al*. (2013) who recoded a quadratic response on EM when the dietary lysine level in the diet was gradually increased from 2.72 to 9.09 g/kg of diet for Dekalb White hens from 37 to 48 wk of age. Bonekamp *et al.* (2010) recorded a non-linear increase in EM with increasing intake of true faecal digestible (TFD) lysine in light hens. Schutte and Smink (1998) also reported a quadratic effect on EM when fecal Dlys intake was gradually increased from 539 to 847 mg/h/d, and suggested 830 mg/h/d of intake of total lysine for the daily production of 50 g of EM. Figueiredo *et al*. (2012) reported a maximum EM of 54.5 g at 780 mg/h/d intake of Dlys. In the current study, a peak EM of 57.6 g was attained at 836 mg/h/d of Dlys intake, as estimated by using a regression model.

This EM value is in close agreement with an average 58.1 g shown for Lohmann-LSL Lite hens from 27 to 66 wk of age (Lohmann-LSL Lite Management Guide, 2013).

3.5.3. Feed intake and feed efficiency

The analysis of FI variance showed a quadratic response to increasing intake of Dlys (Table 3.3). Prochaska *et al*. (1996) also recorded a similar response for FI when Hy-Line (W-36 strain) hens consumed a range of Dlys (638 to 1165 mg/hen/day) for 15 weeks starting at 23 wk of age. The FI response of the current study is also supported by Filho *et al.* (2010) who also reported a positive quadratic increase in FI when graded levels of digestible lysine (6.0, 7.0, 8.0 and 9.0 g/kg of diet) were fed to light laying hens. The reduction in FI for the hens consuming low Dlys diets in the present experiment may be due to lesser nutrient requirements associated with low egg production. Feed intake gradually increased from 651 mg intake level of Dlys until it reached a plateau at 107.2 g/h/d at 795 mg. Bonekamp *et al*. (2010) has also documented similar results. In contrast, Figueiredo *et al.* (2012) reported a decline in FI when the dietary Dlys level was gradually increased from 0.675 to 0.879%. No explanation was given for this response, but it may be due to AA imbalance as the experimental diets were not formulated for a balanced protein. In another study, FI was not impacted when CP in the hen diets was decreased from 16.5 to 14.0% while maintaining constant levels of essential amino acids (Silva *et al*., 2006).

The effect of Dlys intake levels on FE was strongly quadratic. In the current study, a minimum FE response was obtained at 839 mg/h/d of Dlys intake, as estimated by using the quadratic equation, which is similar to the report from Bonekamp *et al.* (2010) who estimated an intake of 800 mg of TFD lysine resulted in the most efficient feed conversion in light hens. Some other researches have reported comparatively lower Dlys requirement for a minimum FE. For example, Schutte and Smink (1998) achieved a best FE at 720 mg/h/d intake of apparent faecal

digestible lysine in White Leghorn hens (exponential regression method). A similar report was presented by Bregendahl *et al.* (2008) when they used the broken-line regression method of nutrient estimation and predicted an intake of 690 mg of true digestible lysine to achieve the best feed conversion ratio. Nathanael and Sell (1980) did not see any improvement in FE when daily lysine consumption was gradually increased from 588 mg to 836 mg, but the treatment diets were not ideally balanced for amino acids and that might have been the reason for seeing no effect. Most research studies have showed an improved feed conversion response with increasing Dlys intake levels (Wu *et al*., 2005; Figueiredo *et al.*, 2012). The current study finds that the hens need a higher level of Dlys for a maximum feed efficiency response in comparison to the requirement for egg production.

3.5.4. Egg size classification

Depending upon their weights, the table eggs are classified for their sizes which is legally required from a marketing and consumer perspective. In the current study, the egg sizes were significantly affected by Dlys intake levels (Table 3.4). Not many studies have looked into this parameter of egg production before. In the commercial egg market, the demand for medium and small size eggs is much less compared to larger eggs. Thus, from an economic point-of-view, it would be more desirable for an egg producer that the hens start laying larger size eggs sooner in the first phase of the egg cycle. Based on this this report, it is possible that the production of smaller eggs can be reduced by manipulating the AA-balanced Dlys intake level, and that may increase the farm revenue. On the other hand, the egg sizes can be controlled at a desired level towards the last phase of egg production when egg sizes tend to increase with reduced shell quality by reducing the AA-balanced Dlys intake of the hens. However, the farm income should always be compared with the cost of egg production to keep the egg operation profitable.

3.5.5. Regression analysis of key parameters

A non-linear regression equation ($y = ax^2 + bx + c$; where y = dependent variable, x = independent variable (Dlys level) and c = intercept), was fitted in a quadratic exponential response curve to estimate the Dlys intake levels for a maximum response on HDEP, EW and EM, and a minimum response on FE in this experiment. Minimum and maximum x values of the regression equation ($ax^2 + bx + c = 0$) were determined and the mean of the two x values was considered as the Dlys requirement, as a balanced protein, to attain the maximum or minimum response of the assessed variable. In the current study, a non-linear regression method was preferred over broken-line method because the latter might underestimate the Dlys requirement. The non-linear regression model has also been criticised for it's over estimation of a nutrient requirement. However, the regression model permits prediction of the response of criteria to Dlys and the economic estimation of the optimum level for specific production conditions. This can be accomplished by comparing the diet costs to the value of eggs produced. Robbins *et al.* (2006) has also suggested that non-linear models are preferred over broken-line or linear model methods for estimating a nutrient requirement in poultry. In this experiment, the non-linear regression model has estimated daily intake of Dlys as a BP for a maximum output of HDEP, EW, EM, and for a minimum FE as 769, 903, 836 and 839 mg/h/d respectively. By using the quadratic function, minimum and maximum x values were calculated, and then a mean of the two extreme x values was considered as Dlys intake requirement for maximum and minimum responses. Digestible lysine requirement estimates from previous studies to reach the highest and most feed efficient egg production are shown in Table 3.5. The data in the table clearly shows that how the Dlys (BP) requirement has increased over the years for a maximum egg and feed response.

Table 3.5 Studies showing daily digestible lysine (Dlys) intake levels required for maximum response of egg production, egg weight, egg mass and feed efficiency

			Trial period	Dlys intake level (mg/h/d)			
Authors	Year	Hen Breed	(wk of age)	HDEP[1]	EW	EM	FE
Schutte and Smink[2]	1998	White Leghorn	24 - 36 wk	539	594	540	720
Bregendahl *et al.*[3]	2008	Hy-Line	28 - 34 wk	482	649	538	693
Bonekamp *et al.*[4]	2010	Lohmann LSL	24 - 60 wk	600	750	700	750
Figueiredo *et al.*[5]	2012	Hy-Line	42 - 58 wk	688	766	780	805
Van Krimpen *et al.*[6]	2015	Laying hens	----	810	----	820	825
Current study[7]	2016	Lohmann LSL	27 - 66 wk	769	903	836	839

[1] HDEP - Hen-day egg production; EW - Egg weight, EM - Egg mass, FE - Feed efficiency; [2]Non-linear regression method; [3]Broken-line regression method; [4]Linear and exponential regression methods; [5]Quadratic and adjusted linear regression method; [6]Meta-analysis study; [7]Non-linear regression method.

It is evident that the daily demand for lysine and other AA by laying hens has been steadily increasing with the increase in their production capacity. Some of the previous studies have reported a much lower Dlys requirement for a highest egg production (Nathanael and Sell, 1980; NRC, 1994; Prochaska *et al*., 1996) based on total lysine requirements. Similarly, Schutte and Smink (1998) and Bregendahl *et al.* (2008) recommended 539 mg (apparent fecal digestible) and 538 mg/h/d (true digestible), respectively. But, the CVB (1996) recommended 700 mg/h/d of Dlys for a maximum egg response which is in close agreement with the findings of the current study (769 mg/h/d). Some of the older studies show much lower Dlys requirements because the level of egg production was also low. Gunawardana *et al*. (2008) did not find an increase in egg production when hens consumed 849, 924 or 1023 mg of total dietary lysine per day in seven brown layer strains from 21 to 36 wk of age, but this may be due to the tested levels being in excess of the requirement.

3.6. Conclusion

According to the results of this study, a daily intake of 769, 903, 836 and 839 mg Dlys as a balanced protein per day are required to maximize HDEP and EW, EM, and for a minimum FE, respectively. This does not imply that these levels are optimum for all production conditions. Using the regression equations for these traits, feed cost and the egg size classification response, it is possible to estimate the economic optimum BP Dlys intake for specific market conditions. These data may provide nutritionists with information required for the economic evaluation of BP levels in laying hen diets.

4. ASSESSING THE RESPONSE OF HEN WEIGHT, BODY COMPOSITION, FEATHER SCORE, EGG QUALITY AND LEVEL OF EXCRETA NITROGEN CONTENT TO THE AMINO ACID-BALANCED DIGESTIBLE PROTEIN INTAKE OF LAYING HENS

4.1. Abstract

Laying hen diets with ideally balanced protein are critical for egg production and feed efficiency, and also impact other important characteristics of laying hens. A study was conducted using cage-housed Lohmann-LSL hens (27 to 66 wk of age) to study the response of non-production characteristics to graded levels of AA-balanced digestible lysine (Dlys) intake (550, 625, 700, 775 and 850 mg/hen/d). Each treatment was replicated 10 times with two cages of six hens per replicate. Data collection included hen weight and feathering (27, 47, 66 wk of age), tissue weights (66 wk of age), egg specific gravity (every three wk), egg component weights (41, 52, 65 wk of age) and excreta nitrogen (N) content (47 and 57 wk of age). The experiment was a completely randomized design and the level of significance was fixed at $P \leq 0.05$. Hen weight increased with increasing Dlys intake in a quadratic fashion. Pectoralis muscle (absolute, % - Q) and abdominal fat pad (absolute - L; % - Q) also increased with amino acid intake. Proventriculus absolute weight increased (L), and gizzard absolute and proportional weights decreased (L) with increasing Dlys intake. The effect of Dlys on small intestine measurements varied by section, but in general absolute and % weights decreased in either a Q or L fashion with increasing Dlys intake. Caeca length and weight were minimally affected by diet. Heart, liver, kidney and ovary weights were unaffected by treatment. Overall bird feather cover increased (L) with increasing Dlys intake. Egg shell quality as indicated by ESG and percent

shell decreased (L) with increasing diet AA level intake. The proportion of albumen and yolk in eggs responded quadratically to Dlys intake, with highest values for albumen and minimum values for yolk at 850 mg Dlys intake. Excreta N content increased quadratically with Dlys intake. In conclusion, digestible balanced protein intake significantly affected hen body weight and composition, and feathering as well as egg quality and excreta N content.

Key words: hen weight, body composition, plumage, egg quality, excreta nitrogen

4.2. Introduction

Laying hens continue to change in many aspects of egg production, largely as a result of genetic selection (Anderson *et al*., 2013), and these changes affect their nutrient requirements. At the same time that production of egg mass has increased, body weight has decreased, thereby improving feed efficiency and also affecting the allocation of nutrients for maintenance and egg production. Among the more important nutrients are the essential amino acids required for both maintenance and production characteristics (Bregendahl *et al.*, 2008). Previous work in this laboratory has examined the effects of balanced digestible amino acids on primary egg production characteristics (Chapter 3), and this work will report on the response of other relevant laying hen characteristics including hen weight and body composition, feathering, aspects of egg quality and the nitrogen content of hen excreta.

Hen weight (HW) is an important characteristic because it impacts feed intake, feed efficiency and egg characteristics (Harms *et al*., 1981). During the first year of an egg production cycle, hens typically gain approximately 0.4 kg, with most of the gain occurring prior to 30 wk of age (Lohmann-LSL Lite Management Guide, 2013). The amount and rate of gain, as well as body weight maintenance, are strongly affected by the dietary supply of protein or AA (Novak *et al*., 2004, 2006; Wu *et al*., 2005; Mousavi *et al.*, 2013). Dietary protein is key for synthesis of tissue proteins and intake levels may affect muscle deposition. Bonekamp *et al.* (2010) reported that the weight gain of heavy and light hens was affected quadratically with increasing intake of balanced protein. In that study, hens consuming the lowest amount of true faecal digestible (TFD) lysine lost body weight.

Hen body composition characteristics, such as abdominal fat pad and skeletal muscle are a reflection of HW and nutrient partitioning. Protein accretion is necessary for growth and

maintenance of skeletal muscles and other body tissues of laying hens (Muramatsu *et al.*, 1987). Lysine and methionine deficiency reduces protein synthesis rates in the liver, oviduct and the whole body of laying hens (Hiramoto *et al.*, 1990). Apart from body tissue growth and maintenance requirements, hens in lay need additional protein or AA for egg production. When animals are fed diets deficient in protein or AA, they can catabolize and deplete their own muscle tissues to obtain necessary AA to maintain their vital physiological and reproductive functions (Pomar *et al.*, 1991). When dietary AA are in excess, they are deaminated in the liver and the carbon fraction of the amino acids can be utilized as energy, and if in excess of the bird's requirements, will be stored as body fat through the process of lipogenesis.

Mature feathers do not have maintenance requirement for nutrients *per se,* but they may account for 20-30% of bird's total body protein (Griminger and Scanes, 1986). Hens do show nutritional and behavioural responses to dietary protein or AA deficiencies in the form of feather pecking and cannibalism (Van Krimpen *et al.*, 2005). Feather pecking and cannibalism are vices that negatively affect bird welfare and are influenced by hen strain, and its environment and nutrition (Savory, 1995). Other than the negative impact on welfare of birds, feather loss has been linked to increased feed intake, which occurs to compensate for the heat loss from exposed body surface (Peguri and Coon, 1993) and this has economic consequences because of increased expenditure for feed. Mature feathers are avascular, but feather condition is influenced by dietary protein and AA levels (Twinning *et al.*, 1976; Tauson and Svenson, 1980) as a result of feather pecking behaviour.

From the consumer perspective, external (egg shell) and internal (albumen and yolk) qualities are of great importance. Since dietary protein or AA levels influence egg size (Chapter 3.0, Table 3.4; Mousavi *et al.*, 2013), it is very likely that they may further impact egg

component measurements. Egg shell quality can be evaluated in different ways such as egg shell weight (absolute and proportional), egg shell thickness, egg shell breaking strength and egg specific gravity. Some previous studies have shown that the absolute (Wu *et al*., 2007) and proportional (Novak *et al*., 2004; Rocha *et al*., 2009; Mousavi *et al*., 2013) egg shell weights do not change with an increase in dietary protein or AA intake. However, Gunawardana *et al*. (2008) reported a decrease in proportional egg shell weight when dietary lysine level was increased from 0.747 to 0.917%. Similarly, egg shell thickness has been shown to decline with increased egg size resulting from the higher intake of AA (Mousavi *et al.*, 2013). Egg specific gravity similarly declines with an increase in egg weight (Wu *et al*., 2007). Keshavarz (2003b) has suggested that the egg shell quality can be improved in older hens by controlling the egg size using dietary manipulation. Absolute internal egg component (albumen and yolk) weights increase with egg size and this is a consistent finding of increasing dietary protein or amino acids in laying hen research (Prochaska *et al*., 1996; Novak *et al*., 2004). However, proportional weights of egg shell, albumen and yolk have generally not been affected by dietary protein or amino acid levels (Yakout *et al*., 2006; Rocha *et al.*, 2009; Figueiredo *et al.*, 2012; Santos *et al.*, 2014). An exception is Gunawardana *et al*. (2008), who reported an increase in absolute and proportional albumen weights with increasing dietary lysine levels (0.747, 0.828 and 0.917%).

Albumen height and Haugh units have been considered important parameters for assessing the internal egg quality (Roberts, 2004). Both methods measure albumen viscosity. Albumen height is considered a more relevant tool to monitor the freshness level of eggs compared to Haugh units as the latter itself is dependent upon albumen height (Silversides, 1994). Albumen quality also declines with hen age (Silversides and Scott, 2001; Roberts and Ball, 2004) and is affected by the genetic selection or strain of the birds (Scott and Silversides, 2000).

Hens are unable to utilize 100% of the dietary protein or AA they consume. The undigested protein can be fermented by anaerobic bacteria in the distal ileum and or caeca or excreted. Upon absorption, the amino acids are mainly utilized for growth, maintenance and egg production, and the un-utilized or excess AA are deaminated in the liver. Nitrogen from the deamination process is mainly excreted as uric acid. Studies have shown that excreta N content increase with increasing intake of dietary protein (Blair *et al*., 1999; Latshaw and Jhao, 2011). Novak *et al*. (2006) successfully reduced N excretion in hens when daily protein intake was reduced from 18.9 to 17.0 g from 20 to 43 wk of age and 16.3 g to 14.6 g/d from 43 to 63 wk of age. Since more synthetic essential AA are available, it has become possible to formulate layer diets with adequate supply of balanced protein to the hens while maintaining a minimum dietary crude protein level.

Generally, the lysine level fed to a laying hen is based on parameters which are of highest economic importance such as egg production, egg weight and feed efficiency. However, other relevant criteria can also be affected, and it is of value to understand how these characteristics respond to the level of dietary digestible amino acids. The objective of this study was to assess the effects of feeding laying hens graded intake levels of amino acid balanced digestible lysine (digestible balanced protein) on hen weight, body composition and feathering, egg component measurements, and excretion of nitrogen. Data in this research was collected simultaneously with production results reported in Chapter 3.

4.3. Materials and methods

The Animal Care Committee of the University of Saskatchewan approved this animal experimentation and the experiment was conducted following the guidelines of the Canadian

Council of Animal Care (1993) as specified in the Guide of the Care and Use of Experimental Animals.

4.3.1. Birds and care

Lohmann-LSL Lite pullets were kept in litter floor pens from hatch until housing in cages at 17 wk of age. The pullets were beak treated at the hatchery and management during the rearing period generally followed the primary breeder's recommendations (Lohmann-LSL Lite Management Guide, 2013). At adult housing, pullets were randomly housed in conventional wire cages (Specht) with cage dimensions of 60.96 cm (length) x 39.37 cm (width) x 40 cm (height). Feed and fresh water was available *ad libitum*. One nipple drinker per cage (shared) was available to provide drinking water. A minimum barn temperature of 20 to 22°C was maintained prior to and during the experiment. A photoperiod of 14 hours with 10 lux of light intensity (at feeder level) was provided by incandescent bulbs. Hens were fed a commercial laying hen ration prior to the start of the experiment, which started at 27 and concluded at 66 wk of age. At the start of the experiment, mean HW was 1,640 g and the HDEP was 97%. Mortality was lower than the breeder standard prior to the start of the research. The experiment was a completely randomized design, and a total of 600 birds were equally distributed in five dietary treatment groups consuming diets providing 550, 625, 700, 775 or 850 mg/hen/day of Dlys. Each treatment was assigned to 10 replicates with each replicate consisting of two adjacent cages with six hens per cage.

4.3.2. Feeds and feeding

Initially, two isocaloric (2,900 kcal/kg of diet), soybean meal (45.09% CP) and wheat (17.29% CP) based diets were constituted in a mash form (Chapter 3, Table 3.1). The base diets contained 500 mg or 850 mg of Dlys/100 g of diet. The base diets were balanced for isoleucine

(79%), methionine (47%), total sulfur AA (94%), threonine (74%), tryptophan (22%) and valine (88%) relative to Dlys level according to the recommendations of Bregendahl *et al*. (2008) and Lemme (2009). The five diets were prepared by mixing the two base diets in calculated proportions based on treatment FI during the preceding three week period (Chapter 3). The diets were formulated with slightly higher ME than the breeder's recommendation to ensure that the energy was not limiting and the dietary protein not utilized for energy purposes.

4.4. Data collection

4.4.1. Hen weight and feather score

Individual hen weight was measured at the beginning (26 wk of age), middle (47 wk of age) and at the end (66 wk of age) of the experiment. At the time of weighing, each hen was scored independently for feather cover by two trained individuals using the method described by Davami *et al.* (1997). The mean of the scores for both the scorers was used in the statistical analysis of the scores from each area of the hen's body. Feathering was assessed on five areas of the body (neck, breast, wings, back and vent) and scored from one to four, with one being featherless and four being fully feathered. The maximum score for a fully feathered hen was 20 points.

4.4.2. Egg specific gravity and egg component measurements

Every three weeks, all the eggs from a day's full production were tested for specific gravity using the floatation method (Holder and Bradford, 1979). The eggs collected from each replicate cage were individually numbered and weighed to the nearest one decimal point. Then eggs were transferred into a wire basket and dipped sequentially in nine different tubs containing a salt solution with a specific gravity of 1.065, 1.070, 1.075, 1.080, 1.085, 1.090, 1.095, 1.100 or 1.110. The eggs floating in each tub were removed and recorded for their specific gravity.

The internal and external egg components; AH, AW, YW and dry ESW were measured at wk 41, 52 and 65 wk of age. Each egg from a day's full production was individually weighed. Then the egg was broken on a glass platform and measured for its albumen height (mm) at the mid-point between the yolk and edge of the thick albumen, by using a TSS QCM Plus instrument (Technical Services and Supplies, Chessingham Park, York, YO19 5SE, United Kingdom). Subsequently, the albumen and yolk were separated using a funnel and weighed. The egg shells were weighed to the nearest one decimal point after 24 h of drying at room temperature.

4.4.3. Body composition and organ measurements

At the end of the experiment, one bird from each of ten replications was randomly selected and humanely euthanized by intravenous injection with 0.5 to 1.0 ml of T-61 Euthanasia Solution (Merck Animal Health, Intervet Canada Corp., 16750 Route Transcanadienne, Kirkland, Canada). The hens were dissected, and pectoralis muscle (major and minor separately), abdominal fat pad, liver, heart and kidneys collected and weighed. Gastrointestinal tract segments were also removed, emptied and weighed; lengths were measured for the duodenum, jejunum, ileum and caeca. The ovary was removed and weighed. The number and size (by diameter) of large yellow follicles (LYF) and small white follicles (SWF) were also recorded.

4.4.4. Excreta sampling for determining total N content

Excreta samples were collected for a period of 24 h from five replicates per treatment at wk 47 and 57 of age to determine excreta nitrogen content. Upon collection, the excreta samples were stored in a freezer at -20°C in plastic bags. The samples were then dried in a forced air oven at 50°C for 72 h, ground using a Retsch mill (Retsch GmbH, Retsch-Allee 1-5, Haan, Germany; 1 mm size screen-hole size) and stored in plastic vials at room temperature. The excreta samples

were then analysed for total N content using the combustion method of Association of Official Analytical Chemists (AOAC, 1995).

4.5. Statistical analysis

The experiment was a completely randomized design. The response means were obtained with one-way ANOVA by using PROC mixed of SAS 9.3. The analyses were completed by using PROC REG (regression) and PROC RSREG (response surface regression) of SAS 9.3 (2003). Each replicate cage was the experimental unit for analysing HW and FS variables. For analysing the data for body composition and organ measurements, the individual hen was considered as an experimental unit and each hen was a representative of its replicate cage. All eggs from each replicate were considered as an experimental unit when analyzing egg specific gravity, AH and egg component measurements. The level of significance was fixed at $P \leq 0.05$.

4.6. Results

Hen weight was not different for treatments at the beginning of the experiment (Table 4.1). At both 47 and 66 wk of age, HW increased with Dlys intake in a quadratic fashion. At 47 wk, maximum HW was found for the 858 mg/h/d Dlys treatment and at 66 wk maximum weight was found for the 795 and 858 mg/h/d groups. Hens fed 707, 795 and 858 mg Dlys per d gained weight during the trial, while those fed 651 mg/h/d did not gain weight and hens fed 560 mg/h/d lost weight. Abdominal fat pad and pectoralis muscle weights (absolute and proportional) at trial end increased with increasing intakes of Dlys (Table 4.1). With the exception of pectoralis minor weights (percent of HW), which was a linear response, all other effects were quadratic in nature.

Table 4.1 Effect of digestible lysine intake on hen weight and body composition

Parameter	Digestible lysine intake (mg/h/d)[1]					SEM[2]	Regression equation[3]
	560	651	707	795	858		
Hen weight (g)							
27 wk of age	1662[4]	1645	1637	1631	1630	0.06	NS[5]
47 wk of age	1485	1654	1709	1768	1792	0.02	$y = - 0.000004x^2 + 0.006x - 0.724$
66 wk of age	1407	1631	1708	1804	1805	0.02	$y = - 0.000005x^2 + 0.009x - 1.838$
BC[6] absolute weights (g)							
Pectoralis major	87.3[7]	119.6	122.6	123.4	122.1	2.93	$y = - 0.0008x^2 + 1.313x - 386.930$
Pectoralis minor	31.4	42.8	42.43	44.9	45.6	1.08	$y = - 0.0002x^2 + 0.369x - 104.053$
Pectoralis total	118.7	162.5	165.0	168.3	167.6	3.95	$y = - 0.0011x^2 + 1.1682x - 490.980$
Abdominal fat pad	29.0	78.4	73.2	76.3	94.7	4.99	$y = 1.1696x - 404.840$
BC proportional weights							
Pectoralis major	6.4[8]	7.1	7.3	7.2	6.9	0.09	$y = - 0.00003x^2 + 0.043x - 8.604$
Pectoralis minor	2.3	2.6	2.5	2.7	2.6	0.03	$y = - 0.009x - 1.114$
Pectoralis total	8.6	9.7	9.8	9.9	9.5	0.12	$y = - 0.0000343x^2 + 0.05192x - 9.719$
Abdominal fat pad	2.0	4.6	4.2	4.4	5.2	0.23	$y = -0.0000376x^2 + 0.06293x - 21.291$

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.
[2] Pooled standard error of mean.
[3]Equations were considered significant if $P \leq 0.05$.
[4] The values are means of the average weights from 10 replications consisting of two adjacent cages with six hens per cage.
[5] NS: Not significant ($P > 0.05$); [5] BC: Body composition.
[7] The values are means of the measurements from 10 hens per treatment at 66 wk of age.
[8] The values are means of the measurements (g/100 g of hen weight) from 10 hens per treatment at 66 wk of age.

Treatment effects on the absolute and proportional weight and length of gastrointestinal tract segments are shown in Table 4.2. Proventriculus and ileum weight increased, while gizzard weight and jejunum length decreased in a linear fashion with increasing Dlys intake. The responses for duodenum length and weight, and ileum length were quadratic with minimum values for the 795, 707 and 795 Dlys intake treatments, respectively. Jejunum absolute weight, and caecal absolute length and weight were not affected by treatment. On a proportional basis, proventriculus and caeca weights were not affected by treatment, but gizzard weight decreased and ileum weight increased in a linear fashion with increasing Dlys intake. The treatment effect was quadratic in nature for duodenum, jejunum and ileum proportional lengths, and for duodenum and jejunum empty proportional weights, with lowest levels at higher levels of Dlys intake. The heart, liver and kidneys weights were not impacted by the treatment, both on an absolute and proportional basis (Table 4.3).

Hens were equally and fully feathered at the beginning of the experiment (Table 4.4). The cumulative FS increased linearly and quadratically with increasing intake of Dlys at 47 and 66 wk of age, respectively. Individually and for all ages, FS for areas of the breast, wings and vent improved linearly, while for the neck and back area the response was quadratic with the maximum score for the 795 and 858 mg Dlys treatments.

Table 4.2 Effect of digestible lysine intake on gastrointestinal tract segment measurements

	Digestible lysine intake (mg/h/d)[1]						
Parameter	560	651	707	795	858	SEM[2]	*P*-Value[3]
Absolute measurements							
Proventriculus weight[4]	5.8[4]	6.6	6.9	6.7	7.3	0.14	0.0007*
Gizzard weight	18.9	17.0	18.7	17.5	15.7	0.36	0.0113*
Duodenum length[6]	24.3	23.6	22.8	22.5	24.2	0.30	0.0390**
Duodenum weight	8.6	8.7	7.3	7.5	8.7	0.21	0.0293**
Jejunum length	57.0	53.4	52.9	48.9	49.8	0.80	0.0004*
Jejunum weight	13.4	14.3	12.9	13.4	14.5	0.36	NS[7]
Ileum length	52.7	49.3	49.6	43.9	48.7	0.72	0.0329**
Ileum weight	9.6	11.6	10.8	11.4	12.6	0.39	0.0199*
Caeca length	26.5	24.9	25.4	22.8	25.0	0.48	NS
Caeca weight	6.7	8.1	7.8	8.2	7.8	0.24	NS
Proportional Measurements							
Proventriculus weight[8]	0.4	0.4	0.4	0.4	0.4	0.01	NS
Gizzard weight	1.4	1.0	1.1	1.0	0.9	0.03	<0.0001*
Duodenum length[9]	1.8	1.4	1.4	1.3	1.4	0.04	0.0004**
Duodenum weight	0.6	0.5	0.4	0.5	0.5	0.02	0.0004**
Jejunum length	4.2	3.2	3.2	2.9	2.8	0.09	0.0016**
Jejunum weight	1.0	0.9	0.8	0.8	0.8	0.02	0.0069**
Ileum length	3.9	3.0	3.0	2.6	2.8	0.09	0.0005**
Ileum weight	0.7	0.7	0.6	0.7	0.7	0.02	NS
Caeca length	2.0	1.5	1.5	1.4	1.4	0.04	<0.0001**
Caeca weight	0.5	0.5	0.5	0.5	0.5	0.01	NS

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.

[2] Pooled standard error of mean; [3]Effects were considered significant if $P \leq 0.05$.

[4] The values are means of the observations from 10 hens per treatment at 66 wk of age.

[5] Absolute weight: g; [6]Absolute length: millimeter; [7]NS: Not significant.

[8] Proportional weight: g/100 g of hen weight.

[9] Proportional length: millimetre/100 g of hen weight; * Linear effect; ** Quadratic effect.

Table 4.3 Effect of digestible lysine intake on heart, liver and kidney weights

	Digestible lysine intake (mg/h/d)[1]						
Parameter	560	651	707	795	858	SEM[2]	*P*-Value[3]
Absolute weights (g)							
Heart	7.1[4]	8.2	7.8	8.0	8.3	0.19	NS[5]
Liver	41.2	54.6	50.6	50.9	52.7	1.69	NS
Kidneys	12.9	15.9	14.7	15.0	15.2	0.46	NS
Proportional weights[5]							
Heart	0.5	0.5	0.5	0.5	0.5	0.01	NS
Liver	3.0	3.3	3.0	3.0	2.9	0.07	NS
Kidneys	0.9	1.0	0.9	0.9	0.8	0.02	NS

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.

[2] SEM: Pooled standard error of mean.

[3]Effects were considered significant if $P \leq 0.05$.

[4] The values are means of the observations from 10 hens per treatment at 66 wk of age.

[5] NS: Not significant.

[6] Proportional weights: g/100 g of hen weight.

Table 4.4 Effect of digestible lysine intake on feather score measured at the start, middle and at the end of the experiment

	Digestible lysine intake (mg/h/d)[1]						
Parameter[2]	560	651	707	795	858	SEM[3]	Regression equation[4]
At 27 wk of age							
Neck	4.0[5]	4.0	4.0	4.0	4.0	0.01	NS[6]
Wings	4.0	4.0	4.0	4.0	4.0	0.00	NS
Back	4.0	4.0	4.0	4.0	4.0	0.01	NS
Vent	4.0	4.0	4.0	4.0	4.0	0.01	NS
Breast	3.9	4.0	4.0	4.0	4.0	0.00	NS
Cumulative score	19.9	19.9	20.0	20	19.8	0.03	NS
At 47 wk of age							
Neck	2.4	2.8	3.1	3.2	3.3	0.08	$y = 0.018x - 4.117$
Wings	3.5	3.7	3.9	3.9	3.9	0.04	$y = 0.010x - 0.009$
Back	2.7	3.1	3.6	3.6	3.7	0.09	$y = 0.021x - 5.017$
Vent	2.0	2.5	2.7	2.8	2.8	0.10	$y = 0.024x - 6.599$
Breast	2.6	2.9	3.0	3.0	3.1	0.07	$y = 0.009x - 0.625$
Cumulative score	13.2	15.0	16.3	16.4	16.8	0.34	$y = 0.082x - 16.368$
At 66 wk of age							
Neck	2.3	2.7	2.9	3.1	3.0	0.05	$y = - 0.00001x^2 + 0.017x - 4.039$
Wings	3.1	3.3	3.6	3.9	3.8	0.05	$y = 0.007x + 0.2679$
Back	2.3	2.8	3.4	3.4	3.5	0.10	$y = - 0.00002x^2 + 0.0315x - 9.133$
Vent	1.4	1.8	2.2	2.1	2.2	0.08	$y = 0.0212x - 6.1324$
Breast	2.2	2.7	2.8	2.9	2.9	0.07	$y = 0.0188x - 4.5006$
Cumulative score	11.3	13.4	14.8	15.2	15.4	0.31	$y = - 0.00006x^2 + 0.096x - 23.537$

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.

[2] Area of the hen body scored for feather cover (scored one to four; one four poorly or no feathers and four for fully feathered); [3]SEM: Pooled standard error of mean.

[4] Equations were considered significant if $P \leq 0.05$.

[5] The values are means of 10 replications consisting of two adjacent cages with six hens per cage.

[6] NS: Not significant.

The egg quality and component measurements are shown in Table 4.5. Increasing Dlys intake resulted in a linear decline in ESG and egg shell weight as a percent of egg weight. In contrast, absolute egg shell weight increased (linear) with increasing Dlys intake. Absolute albumen (linear) and yolk (quadratic) weights increased with increasing intake of Dlys, while proportional albumen and yolk weights were affected quadratically. The latter changes in proportion were quadratic in nature and relatively minor. The ratio of albumen to yolk weight was quadratically related to dietary treatment with minimal ratios for the 707 and 795 mg Dlys intake. Increasing Dlys intake reduced albumen height linearly, but increased the absolute albumen weight in a similar fashion.

The total excreta N content was increased with Dlys intake levels measured at 47 and 57 wk of age, and the overall effect of the treatment was quadratic in nature (Table 4.6).

Table 4.5 Effect of digestible lysine intake on egg specific gravity and the absolute and proportional weights of egg components

	Digestible lysine intake (mg/h/d)[1]						
Parameter	560	651	707	795	858	SEM[2]	Regression equation[3]
Egg specific gravity[4]	1.087[5]	1.087	1.086	1.086	1.854	0.001	$y = -0.00002x + 1.0962$
Albumen height (mm)	8.8	8.9	8.7	8.6	8.6	0.02	$y = -0.0002x + 9.1295$
Absolute weights (g)							
Albumen	31.8	33.9	33.9	35.0	36.3	0.09	$y = 0.029x + 18.904$
Yolk	15.8	17.0	17.2	17.7	17.7	0.05	$y = - 0.00002x^2 + 0.042x + 0.106$
Dry egg shell	5.8	6.0	6.0	6.0	6.1	0.01	$y = - 0.000003x^2 + 0.006x + 3.684$
Proportional weights (%)							
Albumen	56.9	57.0	56.8	56.9	57.9	0.06	$y = 0.00002x^2 -0.026x + 65.356$
Yolk	28.3	28.6	28.8	28.8	28.2	0.05	$y = - 0.00002x^2 + 0.032x + 17.099$
Dry egg shell	10.3	10.1	10.0	9.8	9.7	0.02	$y = - 0.006x + 12.761$
Albumen: Yolk Ratio (g/g)	2.02	2.01	2.00	2.00	2.10	0.01	$y = 0.000002x^2 - 0.003x + 3.122$

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.

[2] SEM: Pooled standard error of mean.

3Equations were considered significant if $P \leq 0.05$.

[4] g/cm^3.

[5] The values are means of 10 replications consisting of two adjacent cages with six hens per cage, considering individual replication as the experimental unit.

67

Table 4.6 Effect of digestible lysine intake on total excreta nitrogen (N) content

	Digestible lysine Intake (mg/h/d)[1]						
Parameter	560	651	707	795	858	SEM[2]	Regression equation[3]
Excreta N content (% DM[4])							
At 47 wk of age	6.12[5]	6.37	6.25	6.95	6.42	0.1118	NS[6]
At 57 wk of age	4.95	5.94	6.29	6.47	5.85	0.2063	$y = - 0.00004x^2 + 0.064x - 17.037$

[1] Means of digestible lysine consumed by treatment replicates from 27 to 66 wk of age.
[2]SEM: Pooled standard error of mean.
[3]Equation was considered if $P \leq 0.05$; [4]DM: Excreta dry matter.
[5] The values are means of five replications consisting of two adjacent cages with six birds per cage; [6]NS: Not significant.

4.7. Discussion

4.7.1. Hen weight, body composition and organ measurements

In the current study, HW increased linearly with increasing intake of Dlys until 47 wk of age, but the overall treatment effect on HW was quadratic. This demonstrates that the length of the experiment is important in interpreting the impact of balanced AA intake on HW. Shorter experiments may not allow sufficient time to demonstrate the full effect. In contrast, longer experiments permit a more complete assessment, but may also be affected by changes in egg mass with age and the subsequent partitioning of AA for production or body weight. Our results are in agreement with the positive effect of balanced protein and amino acids on HW reported in past studies (Parsons *et al*., 1993; Novak *et al.,* 2008; Bonekamp *et al.,* 2010; Shim *et al.,* 2013). On the contrary, Gunawardana *et al*. (2008) did not find any effect on HW when dietary lysine was fed as 0.747, 0.828 and 0.917% of diet from 21 to 36 wk of age to brown egg layers. It is possible in that study that the hens consumed enough lysine even at the lowest lysine level (0.747%) as the average feed intake was 113.6 g/b/d. The observation in the present study that

hens consuming 560 mg of Dlys per day lost weight and that the weight loss continued, although to a lesser degree, during the second half of the experiment is of interest. Despite the loss in weight, these hens continued to lay eggs (general observation and results from tissue hens), albeit at a lower rate and with smaller egg size. Others have similarly shown a loss of BW when fed low levels of protein (amino acids), but not to the same degree as shown in this study (Mousavi *et al.,* 2013). The results from the current study indicate that Lohmann-LSL Lite hens require at least 700 mg/h/d of Dlys to achieve and maintain the desired HW as suggested by primary breeder's recommendation (Lohmann-LSL Lite Management Guide, 2013). Laying hens gain weight during the egg production period and after early growth of reproductive tissues, the gain is mainly due to increased fat deposition. Hens at the beginning of this study averaged 1641 g and growth of reproductive tissue was likely complete or near completion at that time.

Increasing Dlys intake levels increased absolute abdominal fat pad weight linearly, while proportional weights increased in a quadratic fashion. Closer examination of the data shows that both absolute and proportional weights were low for the 560 mg treatment and high for the 858 treatment, with the intermediate levels similar. The increase in fat deposition for the high treatment is likely due to conversion of excess amino acids into glucose through the process of gluconeogenesis in the liver. In turn, glucose would be converted into fatty acids through the process of lipogenesis and deposited as body fat. These data suggest a shift in AA utilization from egg mass to energy storage occurred between a Dlys intake of 795 and 858 mg, which coincides with an estimated maximum egg mass daily intake requirement of 836 mg Dlys (Chapter 3). Hens consuming 560 mg of Dlys had much lower abdominal fat pad weight compared to rest of the treatments at trial end. This is unexpected based on caloric intake of these hens in relationship to egg mass output, which is confirmed by the feed to egg mass ratio

(Chapter 3, Table 3.3). If these values are converted to a calorie to egg mass (g) ratio using diet energy, the values are 6.45, 5.88, 5.61 5.40 and 5.39 calories for the 560, 651, 707, 795 and 858 g Dlys intake treatments, respectively. Chickens fed diets deficient in essential amino acids or protein in relationship to diet energy have been shown to increase fat deposition (Bregendahl *et al*., 2002), unlike what was found in this research. Since excess energy was not stored as adipose tissue, its fate is not clear.

Feathers perform as a natural insulation in birds and help thermoregulation. Reduced feather cover results in increased feed intake (Pigury and Coon, 1993) and heat production. They also reported that the ME utilization (kcal/g of egg mass) was significantly more efficient in hens with 50 or 100% feather cover compared to 0%. Tullett *et al*. (1980) also reported an increase in heat production and a 10% increase in feed intake when the feathers were removed simultaneously from the neck and breast areas of laying hens. In the current study, the hens consumed lowest Dlys had the poorest feather score and possibly they also utilized some of the dietary energy for heat production and thereby reducing fat deposition.

Reduced feather cover is likely part of the answer to increased energy use for egg production due to an increase in hen maintenance requirement, but an effect on other aspects of bird metabolism may warrant consideration. These hens also lost significant amount of pectoralis muscle to meet the AA demand for egg protein synthesis, and these continuous anabolic and catabolic processes could have consumed energy as well. A potential metabolic effect is also supported by research in rats. Rats fed a diet deficient in methionine (0.17%; MR) grow more slowly, consume more food on a gram of body weight basis and have reduced adipose tissue compared to animals fed a methionine adequate diet (0.86%; Malloy *et al*., 2006). Although the exact mechanism is not fully understood, MR feeding impacts a range of important metabolic

mechanisms and increases energy expenditure. Methionine deficiency decreases basal insulin, glucose and leptin levels while increasing adiponectin and triiodothyronine (Carew *et al*., 1997).

Hens with lowest level of Dlys intake had the smallest breast muscle weight at the end of the trial. Based on the loss of body weight in this treatment, it is probable that breast was catabolized to supply AA for egg production. In broiler breeders, a majority of absorbed dietary lysine is incorporated into skeletal muscle, which in turn has been proposed to be critical source of amino acids for egg formation (Ekmay *et al*., 2014). Further, lysine deficiency, as was the case for the 560 mg treatment, has been suggested to enhance muscle synthesis and degradation rates (Tesseraud *et al*., 2001), again potentially affecting bird metabolism and maintenance requirements. It is well established that balanced dietary amino acids and amino acids such as lysine are required for protein synthesis (Hiramoto *et al*., 1990) and skeletal muscle growth (Mac-Donald and Swick, 1981; Muramatsu *et al*., 1987), but the catabolism required for supplying amino acids for maintenance and egg production exceeded the amount available in the diet, and hence the reduced weight. The low breast meat and abdominal fat pad weights were likely responsible for much of the loss of BW noted for the 560 mg Dlys intake treatment.

There was no effect of Dlys intake on liver, heart, and kidney weights and these findings provide evidence that hens fed with the lowest AA deficient diets primarily sacrificed pectoralis muscle to provide AA for egg production and maintained organs important for physiological and reproductive functions. Treatment also did not affect the ovary weight, and the number and size of LYF and SWF. The lack of the effect of treatment on the ovary and follicles is not in agreement with the demonstrated effects of these treatments on egg production and weight (Chapter 3). A lack of adequate replication could be one of the reasons for not seeing a significant effect.

Dietary treatment caused a number of effects on GI tract segment measurements (Table 4.5). Although these effects could be attributed to dietary AA intake, it is possible (and likely probable), that some of the effects relate to the nature of the ingredients used in the diets, which varied in the nature and amount of total dietary fibre in addition to AA content. Absolute proventriculus weight increased as Dlys intake increased, but the proportional weight was unchanged. One can speculate that the effect on absolute proventriculus weight is linked to the increased protein intake in the higher Dlys intake treatments and as a consequence an increased requirement for gastric secretions to digest the protein. In contrast, gizzard absolute and proportional weights decreased with increasing Dlys intake. A possible reason for this effect is the physical nature or fibre content of the experimental diets (Bennett *et al.,* 2002). Similarly decreasing absolute jejunum and ileum lengths could be affected by the nature of diet fibre. Differences in proportional weights are primarily related to differences in hen BW.

4.7.2. Feather Score

Hen feathering improved with increasing Dlys intake, an effect previously demonstrated by other research on dietary protein and amino acids (Neal, 1956; Hughes, 1983; Van Krimpen *et al.,* 2005). Ambrosen and Petersen (1997) reported an improvement in plumage condition when dietary protein was increased from 11.1% to 19.3% in isocaloric diets, with a maximum response observed at 15.2% CP. Mature feathers are avascular structures and do not have nutrient requirement *per se*, so the reduced feathering with lower AA intake is more likely caused by an increase in feather pecking behaviour (Savory, 1995), possibly in order for hens to meet their protein demand. Studies have also shown that a specific AA deficiency, such as lysine and sulfur containing amino acids decreases feather condition. Al Bustani and Elwinger (1987) have demonstrated a significant increase in plumage condition when dietary lysine intake was

increased from 485 to 587 mg/h/d in a low protein diet. A similar AA response on plumage condition was also reported by Elwinger *et al.* (2002) when the lysine and methionine + cysteine levels in organic diets were increased from 5.9 g/kg and 5.1 g/kg to 8.7 g/kg and 6.7 g/kg, respectively. Hughes (1980) reported an increase in nutrient requirement for the hens housed in adjacent cages compared to spaced-caged birds due to greater feather loss by pecking. In the current study, with increasing intake of Dlys FS improved linearly from 27 to 47 wk of age and after that it improved quadratically, possibly due to the reduced AA requirements with age. The hens consumed lowest amount of balanced protein had the poorest feather score mainly due to inadequate AA nutrition, and also possibly due to some behavioural changes as well.

4.7.3. Egg shell quality and egg component characteristics

Egg quality (external and internal) is considered to be highly important from a consumer perspective. In the current study, the egg shell quality was assessed by measuring the absolute and proportional egg shell weights and ESG. Egg shell quality was reduced as the ESW dropped as a percent of EW with increasing intake levels of Dlys. On the contrary, Novak *et al.* (2004), Yakout *et al*. (2006) and Liu *et al*. (2005) reported no effect on percent ESW with increasing lysine levels in their diets. Santos *et al.* (2014) also did not found find an effect on egg shell percentage when the dietary digestible lysine level was gradually increased from 0.600 to 0.900% (digestible lysine intake ranged from 682 to 994 mg/h/d). However, Gunawardana *et al*. (2008), like the current report, also reported a significant decline in percent ESW with increasing dietary lysine levels of 0.747, 0.828 and 0.917% (with average total lysine intake of 1042, 924 and 834 mg/h/d, respectively) in seven strains of brown egg layers. In the current study, egg shell quality declined with increasing egg size. A decline in egg shell quality is a common problem towards the end of the laying cycle, and it is probable that at least a portion of this change is due

to an increase in egg size. These results are also in agreement with the increase in percentage of cracked eggs with increasing Dlys described in Chapter 3 (Table 3.2). Keshavarz (2003b) has suggested that the egg shell quality can be enhanced by controlling the egg size with dietary adjustments with protein being an important component of diet changes.

Egg specific gravity was also affected negatively by the treatment in the current study, most likely due to an increase in egg size with increasing Dlys intake (Chapter 3, Table 3.4). This is in agreement with Wu *et al.* (2007) who also reported a decline in ESG when dietary protein and other EAA density was increased. Other studies (Gunawardana *et al*., 2008; Rocha *et al*., 2009; Santos *et al*., 2014) did not find any impact of lysine levels on ESG. Egg specific gravity is influenced by the proportion of shell weight in relationship to egg weight, and as noted above this value decreased with increasing egg size in the current research.

As expected, albumen and yolk absolute weights increased with increasing egg size associated with Dlys intake. Previous studies (Prochaska *et al*., 1996; Wu *et al.*, 2007 and Gunawardana *et al*., 2008) have also reported similar observations. On a proportion basis, albumen was only changed in a minor way (range – 56.9 to 57.9%). Similarly, the proportional yolk weight was affected quadratically, and again the effect was minor (range – 28.8 to 28.2%). Gunawardana *et al*. (2008) research also supports this finding. Albumen primarily consists of water with albumen solids (majorly albumen proteins) ranging from 11 to 13% while yolk contains some 50% solids (mostly proteins and lipids). Therefore, the amount of available plasma AA can easily impact synthesis of egg proteins and amounts of albumen and yolk weight. Some other studies have shown that lysine intake levels do not impact percentage of egg yolk (Prochaska *et al*., 1996; Figueiredo *et al.*, 2012; Santos *et al*. (2014). The albumen: yolk ratio

was affected quadratically in the current study with increasing Dlys intake, however, the change was very small (range – 2.02 to 2.10).

Albumen height decreased with increasing levels of Dlys intake, a similar effect was reported by Leeson and Caston (1996) when they increased the dietary protein of hen diets from 14.4 to 16.8%. Hammershoj and Kjaer (1999) also reported a decline in albumen height with increasing dietary protein. Albumen is majorly consisted of water with 10-12% of protein solids (Romanoff and Romanoff, 1949). Ovomucin, a sulfated glycoprotein in albumen, composition primarily differ in thin and thick layers of albumen. Ovomucin is responsible for giving albumen a gel like structure and the thick albumen has four times more ovomucin compared to thin albumen. Any changes in ovomucin can impact the viscosity of the albumen. Albumen thinning has also been reported due to interaction of ovomucin with lysozyme, another albumen protein, by affecting the sulfated bond of ovomucin (Robinson and Monsey, 1972). It may be possible that higher protein intake by the hens influences the interaction between ovomucin and lysozyme resulting in thinning of albumen.

4.7.4. Total excreta nitrogen content

The level of nitrogen in excreta in the present study increased with Dlys intake in a quadratic fashion. This corresponds with the protein level of the diet and is in agreement with other work showing a positive relationship between diet protein and excreta nitrogen content (Summers, 1993; Blair *et al*., 1999, Latshaw and Jhao, 2011). However, other reports have indicated a linear not quadratic response. The difference in response could be related to the dietary structure of the experimental diets and the level of amino acids used. The diets in the current study were based on the levels of ideally balanced digestible AA while most previous experiments used CP levels in their diets. Excreta N levels in this work are 6.12, 6.37, 6.25, 6.95, 6.42, and 4.95, 5.94, 6.29,

6.47 and 5.85% of excreta DM at 47 and 57 wk of age, respectively. The effect could have been a linear one if the Dlys intakes were at lower level. In the current study, it was also possible that more N was excreted as ammonia in the excreta of highest Dlys group compared to other groups resulting in lower total detectable N content. Latshaw and Zhao (2011) have found a linear increase in excreta N content when laying hens consumed protein 13, 15 and 17 g per day with mean values of 3.98, 4.85 and 5.68% (% of excreta DM) respectively. Hens can utilize approximately 40% of the total protein consumed. Birds do not have techniques to store excessively digested proteins or AA and the excess protein is excreted from the body as nitrogenous compounds, mainly as uric acid, and also as urea and ammonia (Goldstein and Skadhauge, 2000). Ishibashi (1996) studied the effect of increasing dietary CP from 14.0 to 19.8% in laying hens for 10 days and reported an increase in N excretion. Nitrogen is water soluble and can easily pollute the soil. Poultry manure can be a good source of nitrogen and phosphorus and can be easily utilized to improve the soil fertility for agricultural crops (Preusch *et al*., 2002). Nitrogen excreted as uric acid can be readily fermented by microbes in the litter, under suitable conditions of temperature and moisture, into ammonia and water. Ammonia is not a green-house gas, however, it can pollute the poultry barns and the environment producing harmful health effects on birds and people.

4.8. Conclusion

In addition to performance characteristics, DBP in hen diets can impact other characteristics that indirectly or directly have economic and welfare implications. Characteristics like hen body condition, shell quality and feather cover affect reproductive capacity, the number of marketable eggs, and feed intake, all important in determining the economics of egg production. Poor body condition and feather cover also have welfare implications as they impact hen well-being.

Additional data on excreta nitrogen content is useful in optimizing the use of manure as fertilizer and reducing its environmental impact. Therefore, understanding the response of these characteristics provides a broader understanding of the importance amino acid nutrition in laying hens. It also emphasizes the need for poultry nutritionists to consider factors which are of socio-economic and environmental importance when balancing hen diets for protein and amino acids.

5. OVERALL DISCUSSION AND CONCLUSION

Genetic selection continues to change the nature of the laying hen. These changes include increased egg production and egg size, improved feed efficiency and reduced body weight. In response to these changes, hens must have altered their requirements for nutrients. Since production (egg mass) increases require protein synthesis, the intake of EAA has to increase, and therefore just as this work has done, the nutrient requirements of hens require periodic re-examination.

The results of this experiment suggest that the levels of EAA (BP) required for a maximum response (egg production, egg weight, egg mass and feed efficiency) have increased, which is in agreement with the above point. With an aim to improve feed efficiency, the modern hens are comparatively lighter in weight and have less nutrient requirements for maintenance. In that situation, it has become necessary to understand their ideal BP requirement to ensure its adequate supply for a maximum egg and feed performance. Table 3.5 of this document clearly demonstrates that the digestible lysine requirement of laying hens has been increasing continuously over the years. Lysine has been used as a reference amino acid when formulating diets based on an ideal or balanced protein concept and the other EAA are adjusted relative to lysine level. Therefore, it is essential to understand the optimum Dlys requirement for maximum egg and feed responses. In this study, a maximum response on hen-day egg production, egg weight, egg mass and feed efficiency was achieved at 769, 903, 836 and 839 mg/h/d of AA-balanced Dlys, respectively, which are much higher than the recommendations made by the Lohmann breeder company and the NRC (1994). In the current study, hens increased their feed consumption with increasing production of egg mass. A similar response was also reported by

Bonekamp *et al.* (2010). This demonstrates that hens have an appetite for amino acids. This study also reported that BP intake affects egg size. With this information, it is possible to regulate egg size based on market demand and thereby help increase farm revenue. This information can be used to obtain larger eggs early in the egg cycle and minimize the problem of bigger eggs towards the end of the egg cycle.

The data demonstrate that the level of BP can affect hen welfare as well as economic aspects of egg production. It has been reported in the current study that the hens deposited more abdominal fat with higher intake levels of balanced protein. Abdominal fat is linked to hen obesity which can be stressful to the hens and can be associated with metabolic problems such as fatty liver. Feed intake is crucial in maintaining an ideal hen weight, and the feed intake can be affected by multiple factors. It has been generally perceived that hens regulate their feed intake for meeting their energy demand, however that may not be entirely true as reported by Classen (2016). In that report, he has clearly mentioned that the hens are not precisely able to regulate their feed intake in response to changes in dietary energy levels, and some other factors such as hen breed/strain, age and dietary structure also contribute in altering their feed intake. Therefore, controlling the hen weight by altering the dietary energy level should be carefully monitored as the hens may not change their feed intake in the same proportion. The hen response on feed intake is more pronounced when dietary energy level is reduced than it is increased. The dietary AA levels should also be adjusted based on the feed intake to ensure their adequate supply, and the diets should also provide energy adequately to ensure the maximum utilization of the amino acids consumed. Heavier hens consume more feed for their increased nutrient requirements for maintenance. Hens consuming excessive feed also lay much larger eggs, generally with declining shell quality, due to oversupply of protein and AA.

This report also presents that there was a positive correlation between hens' intake level of AA-balanced Dlys and their feather score. Hens fed inadequate protein tend to eat feathers (cannibalism) to meet their nutritional demand for protein (Van Krimpen *et al.*, 2005). Ambrosen and Petersen (1997) have also reported poor feathering in hens consuming inadequate dietary protein. Thus, adequate supply of balanced protein is necessary to address some of the hen welfare issues such as hen obesity and poor feathering. Studies have demonstrated that hens with poor feather condition consume more feed to compensate the energy loss from their bare skin under sub-optimal barn temperatures (Glatz, 2001). Thus, hen obesity and poor feathering can negatively impact the net economic return of the egg production.

Amino acid intake levels of hens influence egg quality (lysine, Gunawardana *et al.*, 2012). This study has also observed that eggs shell quality was reduced, with lowered egg specific gravity and proportional egg shell weight, when hens consumed higher amounts of BP. Balanced protein intake levels also affected the measurements of internal egg components. Albumen absolute weight increased linearly, and albumen and yolk proportional weights were affected quadratically with increasing Dlys intake. Albumen height was reduced with increasing intake of BP, possibly by quality alteration of certain key albumen proteins such as ovomucin. Albumen height has been used to monitor the internal egg quality, which also reduces with egg storage time. Therefore, a balanced supply of EAA is necessary to produce good quality eggs.

Poultry husbandry has been blamed for polluting the environment due to their higher excretion of organic nitrogenous compounds (uric acid, urea, nitrogen). The relationship between Dlys intake level and N excretion levels was quadratic in the current study, however, a linear response was expected. Studies have shown that nitrogen excretion can be reduced by lowering the dietary protein intake of the hens (Blair *et al*., 1999) without affecting the egg production.

However, achieving a maximum egg production response should not be compromised when lowering of the dietary balanced protein is considered in order to reduce N excretion.

Research on the balanced AA requirements of hens requires careful planning to ensure the results provide information that benefits the laying hen industry. For example, the use of a broad spectrum of digestible AA in diet formulation, AA intake vs AA diet level, the size and number of replications, the length of the experiment and the choice of strain affect the value of information gathered. In addition, blending diets to reduce the impact of specific feed ingredients and collection of all relevant data add accuracy and value to the research. One aspect of the current work that would have gained from a different data collection procedure is excreta content. Although, excreta N content has value, assessing the total excreta N in relationship to feed intake would have added value to the results and permitted a more precise estimate of Dlys intake effects on N retention.

The data collected in this work is comprehensive and permits nutritionists to set dietary levels based on their production objectives (egg production, egg size, feed efficiency). These objectives are not always the same. Examining the cost of diets in relationship to the response of key dependent variables should permit calculation of the economic optimum for egg production. Maximum response is not necessarily the goal in egg production flocks.

Despite this additional research on the response of hens to balanced Dlys, research is still required to fully understand other important elements of AA-balanced nutrition for laying hens. Examples of these questions are as follows:

- Do we know enough about the level and nature of protein (NEAA) in the diet? Are all NEAA equal in supplying N for AA synthesis? Do NEAA have other functions that affect the need for them in a diet?
- As more synthetic (crystalline) AA become economically available, can there be a problem with feeding laying hens when significant amounts of EAA are coming from these sources rather than dietary protein?
- Understanding how to feed the very young hen before peak?
- Understanding how to feed older hens as egg mass production declines? In the current work, despite egg production declining, egg mass did not decline.
- When and how do we control egg size in older hens?

These areas of research will become increasing important as the cost and availability of protein for animal feeding declines, and as hens are kept for 80 to 100 wk production cycles without a moult.

6. REFERENCES

Al Bustani, Z. A., and K. Elwinger. 1987. Response of laying hens to different lysine levels. Acta Agric. Scand. 37:27-40.

Ambrosen, T., and V. E. Petersen. 1997. The influence of protein level in the diet on cannibalism and quality of plumage in layers. Poult. Sci. 76:559-563.

Anderson, K. 1979. Some conventional feedstuffs for laying hens. 1. Effects to production and chemical composition of eggs. Swed. J. Agric. Res. 9:29-36.

Anderson, K.E., G.B. Havenstein, P.K. Jenkins, and J. Osborne. 2013. Changes in laying stock performance, 1958-2011: thirty-seven flocks of the North Carolina random sample and subsequent layer performance and management tests. World's Poult. Sci. J. 69:489-513.

ARC. 1981. The nutrient requirements of pigs. Commonwealth Agricultural Bureaux, Slough, England.

Association of Official Analytical Chemists (AOAC). 1995. Official methods of Analysis.16th ed. Washington, DC.

Baker, D. H. 2006. Comparative species utilization and toxicity of sulfur amino acids. J. Nutr. 136:16705-16755.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks post hatching. Poult. Sci. 73:1441-4147.

Ball, R. O., K. L. Urschel, and P. B. Pencharz. 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. J. Nutr. 137:16265-16415

Balnave, D., R. J. Gill, X. Li, and W. L. Bryden. 2000. Responses of Isa-Brown laying hens to a pre-layer diet containing additional calcium and to dietary protein and lysine concentrations during lay. Aus. J. Agric. Res. 51:779-784.

Bennett, C. D., H. L. Classen, K. Schwean, and C. Riddell. 2002. Influence of whole barley and grit on live performance and health of turkey toms. Poult. Sci. 81:1850–1855.

Berres, J., S. L. Vieira, W. A. Dozier III, M. E. M. Cortês, R. de Barros, E. T. Nogueira, and M. Kutschenko. 2010. Broiler responses to reduced-protein diets supplemented with valine, isoleucine, glycine, and glutamic acid. J. Appl. Poult. Res. 19:68-79.

Blair, R., J. P. Jacob, S. Ibrahim, and P. Wang. 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. J. Appl. Poult. Res. 8:25-47.

Bonekamp, R. P. R. T., A. Lemme, P. J. A. Wijtten, and J. K. W. M. Sparla. 2010. Effects of amino acids on egg number and egg mass of brown (heavy breed) and white (light breed) laying hens. Poult. Sci. 89:522-529.

Bregendahl, K., J.L. Sell, and D. R. Zimmerman. 2002. Effect of low-protein diets on growth performance and body composition of broiler chicks. Poult. Sci. 81:1156-1167.

Bregendahl, K., S. A. Roberts, B. Kerr, and D. Hoehler. 2008. Ideal ratios of isoleucine, methionine, methionine plus cysteine, threonine, tryptophan and valine relative to lysine for White Leghorn-type laying hens of twenty-eight to thirty-four weeks of age. Poult. Sci. 87:744-758.

Burley, H. K., P. H. Patterson, and M. A. Elliot. 2013. Effect of a reduced crude protein, amino acid-balanced diet on hen performance, production cost, and ammonia emissions in a commercial laying hen flock. J. Appl. Poult. Res. 22:217-228.

Calderon, V. M., and L. S. Jensen. 1990. The requirement for sulfur amino acid by laying hens as influenced by the protein concentration. Poult. Sci. 69:933-944.

Campbell, R. G., M. R. Taverner, and D. M. Curic. 1984. Effect of feeding level and dietary protein content on the growth, body composition and rate of protein deposition in pigs growing from 45 to 90 kg. Anim. Prod. 38:233-240

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. E.D. Olfert, B.M. Cross, and A.A. McWilliams, ed. Can. Counc. Anim. Care, Ottawa, Ontario, Canada.

Carew, L. B., K. G. Evarts, and F. A. Alster. 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. Poult. Sci. 76:1398-1404.

Carvalho, F., J. H. Stringhini, M. S. Matos, R. M. J. Filho, M. B. Café, N. S. M. Leandro, and M. A. Andrade. 2012. Performance and nitrogen balance of laying hens fed increasing levels of digestible lysine and arginine. R. Bras. Zootec. 41:2183-2188.

Centraal Veevoederbureau (CVB). 1996. Central Bureau for Livestock Feeding. [Amino acid requirements for laying hens and broiler chickens]. Documentation report nr. 18 (in Dutch), Centraal Veevoederbureau, Lelystad, the Netherlands.

Classen, H. L. 2003. Body weight control in laying hens. Zootec. Inter. 4:134-139.

Classen, H. L. 2013. Response of broiler chickens to dietary energy and its relationship to amino acid nutrition. Pages 107-114 in Proc. 24Th Annual Aust. Poult. Sci. Symp., Sydney, Australia.

Classen, H. L. 2016. Diet energy and feed intake in chickens. Anim. Feed Sci. Tech. (in press).

Coon, C., and B. Jhang. 1999. Amino acid for layers examined. Feedstuffs, 71:13-15, 31.

Cupertino, E. S., P. C. Gomes, L. F. T. Albino, J. L. Donzele, H. H. de Carvalho Mello, M. Schmidt, and A. A. Calderano. 2009. Digestible lysine requirements of laying hens from 54 to 70 weeks of age. R. Bras. Zootec. 38:480-487.

Curtis, P. A., F. A. Gardner, and D. B. Mellor. 1985. A comparison of selected quality and compositional characteristics of brown and white shell eggs. Poult. Sci. 64:297-301.

Danner, E., and W. Bessei. 2000. Influence of sulphur amino acid content on performance and feathering of laying hybrids. Proc. 21th World Poult. Sci. Assoc., Montreal, Canada.

Davami, A., M. J. Wineland, W. T. Jones, R. L. Ilardi, and R. A. Peterson. 1987. Effects of population size, floor space, and feeder space upon productive performance, external appearance, and plasma cortisone concentration of laying hens. Poult. Sci. 66:251-257.

Davis, A. J., and R. E. Austic. 1994. Dietary threonine imbalance alters threonine dehydrogenase activity in isolated hepatic mitochondria of chicks and rats. J. Nutr. 124:1667-1677.

Dawkins M. S., C. A. Donnelly, and T. A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427:342-344.

Dean, D. W., T. D. Bidner, and L. L. Southern. 2006. Glycine supplementation to low protein, amino acid-supplemented diets supports optimal performance of broiler chicks. Poult. Sci. 85:288-296.

Ekmay, R. D., C. Salas, J. England, S. Cerrati, and C. N. Coon. 2014. Lysine partioning in broiler breeders is not affected by energy or protein intake when fed at current industry levels. Poult. Sci. 93:1737-1744.

D'Mello, J. P. F., and D. Lewis. 1970. Amino acid interaction in chicken nutrition: 1. Interrelationship between lysine and arginine. Br. Poult. Sci. 11:299-311.

Elwinger, K., R. Tauson, M. Tufvesson, and C. Hartmann. 2002. Feeding of layers kept in an organic feed environment. Proc. 11Th European Poultry Conference, Bremen, Germany.

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

Evonik AMINOdat 4.0. Amino acid database, Evonik Degussa GmbH, Rellinghauser, Essen 45128, Denmark.

Faria, D. E., R. H. Harms, and G. B. Russell. 2002. Threonine requirement of commercial laying hens fed a corn-soybean meal diet. Poult. Sci. 81:809-814.

Faria, D. E., R. H. Harms, and R. S. Antar. 2003. Re-evaluation of the lysine requirement of commercial laying hens in a corn-soybean meal diet. J. Appl. Anim. Res. 23:161-174.

Figueiredo, G. O., A. G. Bertechini, E. J. Fassani, P. B. Rodrigues, J. A. G. Brito, and S. F. Castro. 2012. Performance and egg quality of laying hens fed with dietary levels of digestible lysine and threonine. Arq. Bras. Med. Vet. Zootec. 64:743-750.

Glatz, P. C., 2001. Effect of feather cover on feed intake and production of aged laying hens. Asian-Australasia J. Anim. Sci. 14:553-558.

Goldstein, D. L., and E. Skadhauge. 2000. Renal and extrarenal regulation of body fluid composition. Pages 265-269 in Avian Physiology, ed. G. C. Whittow, Academic Press, San Diego, California.

Griminger, P., and C. G. Scanes. 1986. Protein metabolism. Pages 326-344 in Avian Physiology, ed. P. D. Sturkie, Springer-Verlag press, New York.

Gunawardana, P., D. A. Roland Sr., and M. M. Bryant. 2008. Performance comparison and lysine requirements of seven commercial brown egg layer strains during phase one. Inter. J. Poult. Sci. 7:806-812.

Hammershoj, M. and J. B. Kjaer. 1999. Phase feeding for laying hens: Effect of protein and essential amino acids on egg quality and production. Acta Agric. Scand. 49:31-41.

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

Hardy, P. M. 1985. Chemistry and biochemistry of the amino acids. Pages 6-24 in The protein amino acids. G. C. Barrett, ed. Chapman and Hall, New York.

Harms, R. H., P. T. Costa, and R. D. Miles. 1981. Daily feed intake and performance of laying hens grouped according to their body weight. Poult. Sci. 61:1021-1024.

Harms, R. H., G. B. Russell, H. Harlow, and F. J. Ivey. 1998. The influence of methionine on commercial laying hens. J. Appl. Poult. Res. 7:45-52.

Harms, R. H., G. B. Russell, and D. B. Sloan. 2000. Performance of four strains of commercial layers with major changes in dietary energy. J. Appl. Poult. Res. 9:535-541.

Harms, R. H., and G. B. Russell. 2001. Evaluation of valine requirement of the commercial layer using a corn-soybean meal basal diet. Poult. Sci. 80:215-218.

Harper, A. E. 1958. Balance and imbalance of amino acids. Prot. Nutr. 69:1025-1041.

Harper, A. E., 1964. Amino acid toxicities and imbalances. Pages 87-134 in Mammalian Protein Metabolism (Vol. 2), eds. H. N. Munro and J. B. Allison, Academic Press, New York.

Harper, A. E. 1984. Interrelationships among the branched chain amino acids. Pages 81-99 in Branched chain amino and keto acids in health and disease. Inter. Symp., Gottingen, Basel, Karger

Heger, J. 2003. Essential to non-essential amino acid ratios. Pages 130-124 in Amino Acids in Animal Nutrition, ed. J. P. F. D'Mello, CABI Publishing, Cambridge, MA, USA.

Hiramoto, K., T. Muramatsu, and J. Okumura. 1990. Effect of methionine and lysine deficiencies on protein synthesis in the liver and oviduct and in the whole body of laying hens. Poult. Sci. 69:84-89.

Holder, D.P., and M.V. Bradford. 1979. Relationship of specific gravity of chicken eggs to number of cracked eggs and percent shell. Poult. Sci., 58:250-251.

Hossain, M. A., A. F. Islam, and P. F. Iji. 2013. Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed vegetable or animal protein diets. S. Afric. J. Anim. Sci. 43:208-218.

Hughes, B. O. 1980. Feather damage in hens caged individually. Br. Poult. Sci. 21:149-154.

Hughes, B. O. 1983. The effects of methionine deficiency and egg production on feather loss in caged layers. Br. Poult. Sci. 24:549-553.

Hurwitz, S., Y. Frisch, A. Bar, U. Eisner, I. Bengal, and M. Pines. 1983. The amino acid requirements of growing turkeys 1. Model construction and parameter estimation. Poult. Sci. 62:2208-2217.

Ishibashi, T. 1990. Amino acid requirement of poultry. Jpn. Poult. Sci. 27:1-15.

Ishibashi, T. 1996. Depression of nitrogen excretion in poultry. Pages 9-15 in Jpn. Anim. Husb. Environm. Forum.

Jensen, L. S., V. M. Calderon, and C. X. Mendonca Jr. 1990. Response to tryptophan of laying hens fed practical diets varying in protein concentration. Poult. Sci. 69:1956-1965.

Jurgens, M. H. 2002. Animal Feeding and Nutrition. 9th edition. Pages 25-35 in Review of Nutrients and Digestion – Protein. Kendall/Hunt Publishing Company, Dubuque, Iowa.

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

Keshavarz K., and R. E. Austic. 2004. The use of low-protein, low-phosphorus, and amino acid- and phytase-supplemented diets on laying hen performance and nitrogen and phosphorus excretion. Poult. Sci. 83:75-83.

Keshavarz, K. 2003(b). Effects of reducing dietary protein, methionine, choline, folic acid, and vitamin B12 during the late stages of the egg production cycle on performance and egg shell quality. Poult. Sci. 82:1407-1414.

Kjaer, J. B., and W. Bessei. 2013. The interrelationship of nutrition and feather pecking in domestic fowl (review paper). Arch. Geflugelk, 77:1-9.

Latshaw, J. D., and L. Zhao. 2011. Dietary protein effects on hen performance and nitrogen excretion. Poult. Sci. 90:99-106.

Leeson, S., and L. J. Caston. 1996. Response of laying hens to diets varying in crude protein or available phosphorus. J. Appl. Poult. Res. 5:289-296.

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

Lemme, A. 2009. Amino acid recommendations for laying hens. Lohmann info. 44:9, Oct. 2009. WebMD. lohmann-information.de/content/l_i_44_artikel11.pdf.

Li, F., L. M. Zhang, X. H. Wu, C. Y. Li, X. J. Yang, Y. Dong, A. Lemme, J. C. Han, and J. H. Yao. 2013. Effects of metabolizable energy and balanced protein on egg production, quality, and components of Lohmann Brown laying hens. J. Appl. Poult. Res. 22:36-46.

Lillie, F. R. 1942. On the development of feather. Biol. Rev. 17:247-266.

Liu, Z., G. Wu, M. M. Bryant, and D. A. Roland, Sr. 2005. Influence of added synthetic lysine in low-protein diets with the methionine plus cysteine to lysine ratio maintained at 0.75. J. Appl. Poult. Res. 14:174-182.

Lohmann-LSL Lite Management Guide. 2013. Lohmann Tierzucht GmbH, Am Seedeich 9-11, 24742 Cuxhaven, Germany.

MacDonald, M. L., and R. W. Swick. 1981. The effect of protein depletion and repletion in the muscle-turnover in the chick. Biochem. J. 194:811-819.

Malloy, V. L., R. A. Krajick, S. J. Bailey, G. Hristopoulos, J. D. Plummer, and N. Orentreich. 2006. Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer-344 rats independent of energy restriction. Aging Cell 5:305-314.

Mitchell, H. H. 1964. Comparative nutrition of man and domestic animals. Academic Press, New York.

Mousavi, S. N., S. Khalaji, A. Ghasemi-Jirdehi, and F. Faroudi. 2013. Investigation on the effects of dietary protein reduction with constant ratio of digestible sulfur amino acids and threonine to lysine on performance, egg quality and protein retention in two strains of laying hens. Ital. J. Anim. Sci. 12:9-15.

Muramatsu, T., K. Hiramoto, and I. Tasaki. 1987. Effects of starvation on protein turnover in liver, oviduct and whole body of laying hens. Comp. Biochem. Physiol. B 87:227-232.

Nathanael, A. S., and J. L. Sell. 1980. Quantitative measurements of lysine requirement of laying hens. Poult. Sci. 59:594-597

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. Natl. Acad. Press. Washington, DC.

Neal, W. M. 1956. Cannibalism, pick-outs and methionine. Poult. Sci. 35:10-13.

Newkirk, R. W., and H. L. Classen. 1998. In vitro hydrolysis of phytate in canola meal with purified and crude sources of Phytase. Anim. Sci. and Feed Tech., 72:315-327.

Nichelman, M., E. Branyiova, R. Goli, and B. Tzschentke. 1986. Influence of feather cover on heat balance in laying hens (*Gallus domesticus*). J. Therm. Biol. 2:121-126.

Novak, C., H. M. Yakout, and S. E. Schiedeler. 2004. The combined effects of dietary lysine and total sulfur amino acid level on egg production parameters and egg components in Dekalb Delta laying hen. Poult. Sci. 83:977-98.

Novak, C., H. M. Yakout, and S. E. Schiedeler. 2006. The effect of dietary protein level and sulfur amino acid: lysine ratio on egg production parameters and egg yield in Hy-Line W-98 hens. Poult. Sci. 85:2195-2206.

Olszewski, A. 1978. Competitive inhibition and exchange transport between arginine and lysine in the process of absorption from the caecum in pigs. Acta Physiol. Pol. 29(2):161-165.

Parsons, c. M., K. W. Koelkebeck, Y. Zhang, X. Wang, and R. W. Leeper. 1993. Effects of dietary protein and added fat levels on performance of young laying hens. J. Appl. Poult. Res. 2:214-220.

Peganova, S., and K. Eder. 2002. Studies on requirement and excess of isoleucine in laying hens. Poult. Sci. 81:1714-1721.

Peganova, S., and K. Eder. 2002. Studies on requirement and excess of valine in laying hens. Euro. Poult. Sci. 66:241-250.

Peganova, S., and K. Eder. 2003. Interactions of various supplies of isoleucine, valine, leucine and tryptophan on the performance of laying hens. Poult. Sci. 82:100-105.

Peganova, S., F. Hirche, and K. F. Eder. 2003. Requirement of tryptophan in relation to the supply of large neutral amino acids in laying hens. Poult. Sci. 82:815-822.

Peguri, A., and C. Coon.1993. Effect of feather coverage and temperature on layer performance. Poult. Sci. 72:1318-1329.

Penz Jr., A. M., and L. S. Jensen. 1991. Influence of protein concentration, amino acid supplementation, and daily time of access to high- or low-protein diets on egg weight and components in laying hens. Poult. Sci. 70:2460-2466.

Pesti, G. M. 1991. Response surface approach to studying the protein and energy requirements of laying hen. Poult. Sci. 70:103-114.

Pomar, C., D. L. Harris, and F. Minvielle. 1991. Computer simulation model of swine production systems: II. Modeling body composition and weight of female pigs, fetal development, milk production, and growth of suckling pigs. J. Anim. Sci. 69:1489-1502.

Preusch, P. L., P. R. Adler, L. J. Sikora, and T. J. Tworkoski. 2002. Nitrogen and phosphorus availability in composted and uncomposted poultry litter. J. Environ. Qual. 30:2051-2057.

Prochaska, J. F., J. B. Carey, and D. J. Shafer. 1996. The effect of L-lysine intake on egg component yield and composition in laying hens. Poult. Sci. 75:1268-1277.

Ribeiro, M.L.G., J.H.V. Silva, and F. G. P. Costa. 2002. Effect of lysine levels and protein on the parameters of carcasses and protein content of quail eggs. Rev. Bras. Cienc. Avic. (Supl.), 4:68.

Robbins, K. R., A. L. Saxton, and S. S. Southern. 2006. Estimation of nutrient requirements using broken-line regression. J. Anim. Sci. 84:155-165.

Roberts, J. R. 2004. Factors affecting egg internal quality and egg shell quality in laying hens. Poult. Sci. 41:161-177.

Roberts, J. R., and W. Ball. 2004. Egg quality guidelines for Australian egg industry. Pages 326-330 in Proc. 11Th European symposium on the quality of eggs and egg products, Doorwerth, Netherlands.

Robinson, D. S., and J. B. Monsey. 1972. Changes in the composition of ovomucin during liquefaction of thick egg white. J. Sci. Food Agric. 23:29-38.

Rocha, T. C., P. C. Gomes, J. L. Donzele, S. L. T. Barreto, and H. H. C. De Mello. 2009. Digestible lysine levels in feed for 24 to 40 weeks old laying hens. R. Bras. Zootec. 38:1726-1731.

Roland, D. A. Sr. 1979. Factors affecting shell quality of aging hens. Poult. Sci. 58:774-777.

Romanoff, A. L., and A. Romanoff. 1949. The Avian Egg. John Willey & Sons Company, New York.

Sa, L. M., P. C. Gomes, H. S. Rostagno, L. F. T. Albino, and P. D'Agostini. 2007. Nutritional requirement of lysine for laying hens in the period from 34 to 50 weeks old. R. Bras. Zootec. 36:1829-1836.

Safaa, H. M., M. P. Serrano, D. G. Valencia, X. Arbe, E. Jime´nez-Moreno, R. La´zaro, and G. G. Mateos. 2008. Effects of the levels of methionine, linoleic acid, and added fat in the diet on productive performance and egg quality of brown laying hens in the late phase of production. Poult. Sci. 87:1595-1602.

Santos, T. A. D., A. Geraldo, L. C. Machado, K. Pelicia, S. D. Simao, and J. A. V. Filho. 2014. Digestible lysine levels for laying hens and their effect on egg quality. Acta Scientiarum. Anim. Sci. 36:41-47.

SAS Institute. 2003. SAS/STATS Users Guide. SAS Institute Inc., Cary, North Carolina, USA.

Savory, C. J. 1995. Feather pecking and cannibalism. World's Poult. Sci. J. 51:215-219.

Schutte, J. B., and W. Smink. 1998. Requirement of laying hens for apparent fecal digestible lysine. Poult. Sci. 77:697-701.

Scott, T. A., and F. G. Silversides. 2000. The effect of storage and strain of hen on egg quality. Poult. Sci. 79:1725-1729.

Shim, M. Y., E. Song, L. Billard, S. E. Aggrey, G. M. Pesti, and G. Sodsee. 2013. Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poult. Sci. 92:2687-2696.

Silk, D. B. A., K, G. K. Grimble, and R. G. Ree. 1985. Protein digestion and amino acid and peptide absorption. Pages 63-72 in the 44th Proc. of the Nutrition Society.

Silversides, F. G. 1994. Is the Haugh Unit correction for egg weight valid for eggs stored at room temperature? Poult. Sci. 73:50-55.

Silversides, F. G., and T. A. Scott. 2001. Effect of storage and layer age on quality of eggs from two lines of hens. Poult. Sci. 80:1240-1245.

Sklan, D., and I. Plavnik. 2002. Interactions between dietary crude protein and essential amino acid intake on performance in broilers. Br. Poult. Sci. 43:442-449.

Stadelman, W. J., and O. J. Cotterill. 1995. Egg Science and Technology (4th Edition). Food Products Press, The Haworth Press Inc. New York.

Stettenheim, P. R. The integumentary morphology of modern birds – an overview. Amer. Zool. 40:461-477.

Stilborn, H. L., E. T. Morgan Jr., R. M. Gous, and M. D. Harrison. 1995. Effect of age on feather amino acid content in two broiler strain and sexes. J. Appl. Poult. Res. 6:205-209.

Summers, J. D. 1993.Reducing nitrogen excretion of laying hen by feeding lower crude protein diets. Poult. Sci. 72:1473-1478.

Tan, S. L., and P. J. Parker. 2003. Emerging and diverse roles of protein kinase C in immune cell signalling. J. Biochem. 376:545-552.

Tauson, R., and S. A. Svensson. 1980. Influence of plumage condition on the hen's feed requirement. Swed. J. Agric. Res.10:35-39.

Tesseraud, S., S. Temin, E. Le Bihan-Duval, and A. M. Chagnaeu. 2001. Increased responsiveness to lysine deficiency of pectoralis major muscle protein turn-over in broilers selected on breast development. J. Anim. Sci. 79:227-233.

Tullett, S. G., M. G. McLeod, and T. R. Jewitt. 1980. The effects of partial defeathering on energy metabolism in the laying fowls. Br. Poult. Sci. 21:241-245.

Tuttle, W. L., and S. L. Balloun. 1976. Leucine, isoleucine and valine interactions in turkey poults. Poult. Sci. 55:1737-1743.

Twining Jr, P. V., O. P. Thomas, and E. H. Bossard. 1976. The Number of feathers on the litter; another criterion for evaluating the adequacy of broiler diets. Poult. Sci. 55:1200-1207.

Valério, S. R., R. F. M. de Oliveira, J. L. Donzele, P. C. Gomes, L. R. Apolônio, and W. de O. Resende. 2003. Digestible lysine levels in diets maintaining or not the amino acids relation for broilers from 22 to 42 days of age kept under heat stress. R. Bras. Zootec. 32:372-382.

Van Emous, R. A., R. P. Kwakkel, M. M. van Krimpen, and W. H. Hendriks. 2013. Effects of growth patterns and dietary crude protein levels during rearing on body composition and performance in broiler breeder females during the rearing and laying period. Poult. Sci. 92:2091-2200.

Van Krimpen, M. M., R. P. Kwakkel, B. F. J. Reuvekamp, C. M. C. Van Der Peet-Schwering, L. A. Den Hartog, and M. W. A. Verstegen. 2005. Impact of feeding management on feather pecking in laying hens. World's Poult. Sci. J. 61:663-686.

Van Krimpen, M. M., T. Veldkamp, J.W. van Riel, V. Khaksar, H. Hashemipour, M.C. Blok, and W. Spek. 2015. Estimating requirements for apparent faecal and standardised ileal digestible amino acids in laying hens by a meta-analysis approach. Wagenigen UR Livestock Research report 848:1-72.

Waguespack, A. M., S. Powell, T. D. Bidner, and L. L. Southern. 2009. The glycine plus serine requirement of broiler chicks fed low-crude protein, corn-soybean meal diets. J. Appl. Poult. Res. 18:761-765.

Wu, G. Y. 2009. Amino acids. 37:1-17.

Wu, G., M. Bryant, P. Gunawardana, and D. A. Roland Sr. 2007. Effect of nutrient density on performance, egg components, egg solids, egg quality, and profits in eight commercial leghorn strains during phase one. Poult. Sci. 86:691-697.

Wu, G., Z. Liu, M. M. Bryant, and D. A. Roland, Sr. 2005. Performance comparison and nutritional requirements of five commercial layer strains in phase IV. Inter. J. Poult. Sci. 4:182-186.

Wu, G., M. M. Bryant, R. A. Voitle, and D. A. Roland Sr. 2005. Effect of dietary energy on performance and egg composition of Bovans, White and Dekalb White hens during phase 1. Poult. Sci., 84:1610-1615.

Yakout, H. M., H. Hoehler, and C. Novak. 2006. Effects of reducing dietary protein on performance of White Leghorn layers during the second production cycle. Poult. Sci. 85:124-130.

Appendix 1

Abstract: Poultry Science Association meeting 2014, Corpus Christie, TX (USA).

The digestible lysine requirement of laying hens while maintaining an ideal amino acid profile

D. Kumar*, C. Raginski, K. Schwean-Lardner, and H.L. Classen[1]

University of Saskatchewan, Saskatoon, SK, Canada

As laying hens continue to improve in egg production and other characteristics, it is relevant to examine their response to dietary digestible ideal amino acid level. Research using Lohmann LSL pullets (28 to 40 wk of age) compared digestible lysine (Dlys) intake levels of 550, 625, 700, 775 and 850 mg/hen/d, with Dlys kept in proportion to other essential amino acids. Each treatment was replicated 10 times (2 cages of 6 birds each). Diets containing 500 and 850 mg Dlys/kg feed were blended every 3 wk based on feed intake to produce diets that would provide treatment levels of Dlys intake. Data collection included egg production (5 d/wk), and feed intake, egg weight and egg specific gravity every 3 wk. Feed efficiency (kg feed/kg egg mass; kg feed/dozen eggs) was calculated based on egg and egg mass production, and feed intake. Data were analyzed for Dlys effects using SAS 9.3 and a completely randomized design; regression analysis was used to study the relationship between dependent variables and Dlys intake. Analyses were considered significant when $P \leq 0.05$. In the following results, the impact of Dlys on response criteria will be described in text as well as with bracketed treatment means with means listed in order of increasing Dlys intake (550, 625, 700, 775 and 850 mg/hen/d). Means followed by different superscript letters are significantly different according to Tukey's mean separation test. Actual Dlys intake (mg/d) varied slightly from treatment objectives as shown by the comparative values shown in brackets (550 vs 553; 625 vs 647; 700 vs 719; 775 vs 804; 850 vs 856 mg/d). Hen-day egg production increased in a quadratic fashion with Dlys intake (88.4^{b}, 94.4^{a}, 96.7^{a}, 97.2^{a}, 97.0^{a} %). Egg weight (54.4^{d}, 57.0^{c}, 58.5^{b}, 59.7^{a}, 60.0^{a} g) and egg mass (48.5^{c}, 53.9^{b}, 56.5^{ab}, 58.0^{a}, and 58.0a g/h/d) increased in a similar fashion with Dlys intake. Egg specific gravity (1.089^{a}, 1.088^{a}, 1.088^{a}, 1.088^{a}, 1087^{b}) decreased quadratically with increasing Dlys intake. Feed efficiency (kg feed/kg egg mass – 1.87^{a}, 1.79^{b}, 1.74^{bc}, 1.68^{c}, 1.67^{c}) decreased in a quadratic manner. In contrast, Dlys intake did not affect feed per dozen eggs (1.32, 1.32, 1.32, 1.31, 1.30 kg feed per dozen). In conclusion, the requirement of laying hens for amino acid balanced Dlys varies with response criteria.

Appendix 2

Abstract: Poultry Science Association meeting, 2015, Louisville, KY (USA).

Ideally balanced amino acid levels affect laying hen weight and composition, feathering and amounts of egg components

D. Kumar*, C. Raginski, K. Schwean-Lardner, and H. L. Classen[1]

University of Saskatchewan, Saskatoon, SK, Canada.

The response of laying hens to balanced dietary protein goes beyond the key production characteristics of egg production, egg weight and feed efficiency. A study was completed to assess the response of Lohmann LSL hens (27-66 wk of age) to graded levels of digestible lysine (Dlys) intake (bracketed values corrected for feed intake) of 550 (560), 625 (651), 700 (707), 775 (795) and 850 (858) mg/hen/day, while other essential AA were balanced relative to Dlys level. This report focuses on body weight (BW), tissue weights, feathering quality, and size of egg components as response criteria. A completely randomized design, and Proc Mixed and Proc Reg of SAS 9.3 were used to analyze data. Effects were considered significant when $P \leq 0.05$. BW at the beginning of the trial averaged 1.64 kg and was not affected by treatment. At trial end, the average BW for hens consuming 560, 651, 707, 795, and 858 mg Dlys/d averaged 1.41, 1.63, 1.71, 1.80, and 1.81 kg, respectively. Absolute and proportional fat pad and breast muscle weights increased with Dlys intake, with all responses quadratic except absolute total breast and proportional pectoralis minor weights, where the increase was linear. Ovary weight, number of follicles and size of follicles were not affected by treatment. Overall feathering improved linearly with increasing Dlys intake, as it did for 4 of the 5 areas examined (neck, wings, vent, and breast); back area feathering improved quadratically with Dlys intake. Albumen height (mm) and weight (g) increased linearly with increasing Dlys, while increases in egg shell and yolk weight were quadratic. On a proportion basis, egg shell decreased linearly with increasing Dlys intake. Yolk and albumen proportional weight responded to Dlys in a quadratic manner with highest values found for the 707 and 795, and 858 mg/d treatments, respectively. In conclusion, Dlys levels affect hen body weight and composition, feathering and the amounts of egg components.

Appendix 3

List of presentations, conferences attended and awards

1. **Kumar, D.,** C. Raginski, K. Schwean-Lardner, and H. L. Classen. Protein and amino acid requirements of laying hens. NSERC-IRC (Poultry Nutrition) annual meeting, June 2014
2. **Kumar,** D., C. Raginski, K. Schwean-Lardner, and H. L. Classen. Assessing the digestible lysine requirement of laying hens. PSA annual meeting (Corpus Christie, TX), July 2014
3. **Kumar**, D., C. Raginski, K. Schwean-Lardner, and H. L. Classen. Ideally balanced amino acid levels influence egg performance, feed efficiency, egg quality, feathering, and hen weight and body composition. Annual Prairie Poultry Meeting, May 2015.
4. **Kumar, D.,** C. Raginski, K. Schwean-Lardner, and H. L. Classen. Ideally balanced amino acid levels influence egg performance, feed efficiency, egg quality, feathering, and hen weight and body composition. NSERC-IRC annual meeting, June 2015.
5. **Kumar**, D., and H. L. Classen. Pre-lay nutrition of commercial pullets. An industry report presentation at NSERC-IRC Meeting, June 2015.
6. **D. Kumar**, C. Raginski, K. Schwean-Lardner, and H. L. Classen. Ideally balanced amino acid levels influence egg performance, feed efficiency, egg quality, feathering, and hen weight and body composition. Annual PSA meeting (Louisville, KY), July 2015.
7. Attended Western Nutrition Conference (Saskatoon, Sept., 2013)
8. Attended Poultry Service Industry Workshop (Banff, Sept., 2013)
9. Attended Western Nutrition Conference (Edmonton, Sept., 2014)
10. Attended Poultry Service Industry Workshop (Banff, AB. Oct., 2014)
11. Attended Western Nutrition Conference (Winnipeg, Sept., 2015)
12. Attended Poultry Service Industry Workshop (Banff, AB. Oct., 2015)
13. Received Dave Christenson Travel Award (2014)
14. Received Education Enhancement Award, Dept. of Animal and Poultry Science, University of Saskatchewan (2015)
15. Received J. M. Bell Graduate Scholarship (2015)