

AN ABSTRACT OF THE THESIS OF

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Title: Legume Grains (*Phaseolus vulgaris* and *Pisum sativum*) of the Pacific Northwest as an Alternative Broiler Feedstuff.

Abstract approved:

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Increasing commodity and fuel prices can be a deterrent to growing broilers in the Pacific Northwest. One of the most common protein components of the broiler ration is soybean meal derived from soybeans grown in the Midwestern United States. In an effort to reduce the reliance on this product, alternative local feedstuffs were examined. Three experiments were performed to identify the feasibility of including *Phaseolus vulgaris* (common bean) and *Pisum sativum* (field pea) in broiler rations. The first experiment assessed the 30% inclusion of three different raw beans, pinto, Great Northern white, and small red beans into broiler diets for 42 days. Pinto beans outperformed both white and red beans, but weight gain and feed efficiency of all three were significantly reduced when compared to the corn-soy based control diet. Experiment two compared the growth rates of broiler chicks fed diets containing either 30% heat-treated or 30% raw versions of the three bean cultivars for 21 days. Chick growth was improved by the heat treatment of Great Northern white beans; however, heat treatment demonstrated little or no impact on the birds fed pinto or small red beans. The final experiment assessed the general dietary inclusion of field peas and the alternative feeding practices of sprouting legume grains completely excluding

imported soy products. Including field peas at 30% of the total ration did not inhibit broiler growth when compared to the corn-soy based control diet in the 42 day trial. In a 30% pea diet, sprouting 10% of those peas did not significantly alter chick growth when compared to a standard inclusion of 30% dried field peas; however, feed conversion was significantly improved. A 76% pea diet without soy products significantly reduced feed efficiency and bodyweight gain.

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Legume Grains (*Phaseolus vulgaris* and *Pisum sativum*) of the Pacific Northwest as
an Alternative Broiler Feedstuff

by
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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented July 22, 2009
Commencement June 2010

Master of Science thesis of Sarah-Cate Antoine presented on July 22, 2009.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Sarah-Cate Antoine, Author

ACKNOWLEDGEMENTS

I would like to thank Dr. Hermes for his knowledge and guidance throughout this process. I would also like to thank my parents for their love and support. Their thoughtful advice kept me chasing the proverbial carrot during my journey. The mouse has reached the top step. I offer my appreciation to my friends who listened with patience as I discussed the trials and tribulations of my research, and who inadvertently received a truncated education in poultry management.

My appreciation is offered to Rachel McGovern, Laura Tensa, and Anthony Tautfest, Animal Science students who volunteered their time to assist me with weighing birds, collecting samples, and many other tasks. In addition, many thanks to Margaret Kaaekuahiwi for her assistance, attention to detail, and her constant pursuit for research excellence.

I would like to recognize the Department of Animal Sciences for granting my graduate research assistantship and the C.M. Wilcox Memorial Scholarship for their contributions to my education. Thank you also to Central Bean Company and Union Point Custom Feeds for ingredient acquisition.

TABLE OF CONTENTS

	<u>Page</u>
Chapter 1 Introduction	1
Market Overview	1
Alternative Production	2
Local Procurement	3
Legumes for Sustainability	8
Chapter 2 Literature Review	11
<i>Phaseolus vulgaris</i>	11
<i>Pisum sativum</i>	12
Effects of Legumes on Carcass Quality	15
Antinutritional Factors	15
Protease Inhibitors	16
Lectins	18
Tannins	21
Amylase Inhibitors	24
Phytates	25
Non-starch Polysaccharides and Oligosaccharides.....	28
Alleviation of Antinutritional Factors	30
Soaking and Aqueous Heating	30
Autoclaving	31
Dry Toasting	33
Dehulling	34
Supplementation	34
Germination	35
Extrusion	36
Comparison of Methods	36
Chapter 3 Methods and Materials	38
Experiment One	38
Experiment Two	40
Experiment Three	42
Chapter 4 Results and Discussion	45
Experiment One	45
Experiment Two	49
Experiment Three	52
Conclusion	56
Literature Cited	64

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Ration Formulations of All Diets Tested in Experiments 1, 2, and 3.....	58
2. <i>Phaseolus vulgaris</i> Nutritional Analysis (% DM).....	59
3. <i>Phaseolus vulgaris</i> Amino Acid Analysis (%).....	59
4. <i>Pisum sativum</i> Nutritional Analysis (% DM).....	60
5. Experiment 1: Final Bodyweights (kg) of Broiler Chicks Fed Common Beans on Day 42 Expressed as Replicate and Overall Means.....	61
6. Experiment 1: Mean Feed Conversions of Broiler Chicks Fed Varieties of Common Beans. Days 0 Through 42.	61
7. Experiment 2: Final Bodyweights (kg) of Broiler Chicks Fed Heat-Treated (HT) and Raw Common Beans on Day 21 Expressed as Replicate and Overall Means.....	62
8. Experiment 2: Mean Feed Conversions of Broiler Chicks Fed Varieties of Common Beans Heat-Treated (HT) and Raw. Days 0 Through 21.....	62
9. Experiment 3: Final Bodyweights (kg) of Broiler Chicks Fed Diets Utilizing Field Peas on Day 42 Expressed as Replicate and Overall Means.....	63
10. Experiment 1: Mean Feed Conversions of Broiler Chicks Fed Diets Utilizing Field Peas. Day 0-42.....	63

CHAPTER 1

INTRODUCTION

Market Overview

In the seven day period ending on April 4, 2009, over 156 million broilers were processed in the United States (AMS, 2009a). The U.S. annually produces over 40 billion pounds of poultry meat, approximately 80% of which is broiler meat (ERS, 2009a). These facts offer an indication of the poultry industry's economic importance. While size alone is a sufficient reason to continue research in this area of agriculture, variation among production methods, marketing, and consumption habits constitute the need for further exploration.

There are many important differences between poultry production and other forms of animal agriculture. The vertical integration model used by poultry producers is uncommon for animal production since not only is animal ownership retained throughout the growth period, but often the feedmills, slaughtering/packaging facilities, and trucking fleets are also owned by the same company. Most commercial broilers produced in the U.S. are owned and managed by a small number of industry leaders. This model is responsible for the consistent quality and low price of broiler meat which is not necessarily found in beef, pork, or lamb production. While these attributes are enjoyed by most consumers, there is a growing segment of customers who are in search of alternatively produced poultry. Some are concerned with how the birds are raised, others in what the birds are fed, and some factors in concert along with many others. Fortunately for this market segment, the modern broiler easily

lends itself to production in a variety of environments and is able to utilize many alternative feedstuffs.

Alternative Production

The phrase “alternative production method” represents any style of production that is not typical of the larger commercial industry. In poultry production, examples of alternative production are organic, free range, pastured, antibiotic free, humanely raised, and animals fed specialty or designer diets. Claims of this latter category are economically important as unconventional rearing practices can add substantial value to poultry products and cost to production methods. To illustrate this point, promotional prices for boneless and skinless chicken breasts from 18,600 major supermarkets across the U.S. reported in early March 2009 averaged \$2.75 per pound for conventional broilers, \$4.55 per pound for specialty type broilers (specialty defined as birds fed an all vegetarian diet, no addition of antibiotics, and minimally processed), and \$6.99 per pound for certified organically grown chickens (AMS, 2009b). From the reported prices, value-added labeling implies alternative standards for how the bird was fed, managed, or processed can substantially increase the retail price of poultry meat products. Currently, few of these production techniques and labeling parameters are governed by a regulatory agency. The Food Safety and Inspection Service (FSIS) is responsible for guaranteeing the accuracy and truthfulness of poultry product labeling. Only some “value-added labels” are certified by the FSIS and others are explicitly rejected by the agency for use on poultry

products. Labeling phrases such as “No Chemicals,” “No Antibiotics,” and “No Hormones” cannot be used without specific caveats. For example the claim “No Hormones Added” can be used on poultry, but it must also state that “Federal regulations prohibit the use of hormones,” as not to imply that other producers are supplementing poultry with exogenous hormones. Some labeling phrases, such as: “organic,” “free range,” and “fresh” have a definitive set of standards outlined by the FSIS which are certified by the USDA (FSIS, 2006). The validity of other claims, for example: “humanely raised,” “cruelty free,” “cage free,” and “pastured” present on labels must be deciphered by consumers through an understanding of the practices of various businesses and farms.

Specialty and designer diets are formulated to confer specific traits onto the animals consuming them. For organic diets, the animal (and subsequently its meat) is supposed to be free of antibiotic and pesticide residues. Some specialty diets are alleged to increase the concentration of specific nutritional components, such as the inclusion of flax seed meal in the layer hen diet which increases the concentration of omega fatty acids in the egg (Cherien and Sim, 1991).

Local Procurement

Local procurement is the process of obtaining food, products, and services from local producers. Consumers support local procurement for many reasons: enhancement of the local economy, specialized or hand crafted products, less industrialized monoculture, sustainable production practices, reduced dependence on

nonrenewable energy used for transportation, etc. Consumers define “local” in various ways. For some, it is within a particular city and surrounding area, a radiating distance from their home, state or county borders, a watershed, or ecological zone; for others, it can be defined as simply as products made within one’s own country. While the word “local” can have many definitions, a commonly used description is “within one day’s drive,” about 300 miles.

An emerging term is “locavore” consumers, who undertake the goal of eating only, or primarily, locally produced foods. It has been suggested that some of the driving forces behind this movement are trends found on college campuses, concerns about climate change, recession, the American health food “push”, a resurgence of the family dinner table, and a move toward organics (Mintel Oxygen, 2009). Some college campuses have established models for local procurement using dining halls as examples. As more students are exposed to the idea of eating locally, there will be a larger group of adults who see value in the concept.

One of the strongest ideals behind the locavore movement is reducing one’s carbon footprint. Because the phrase carbon footprint is relatively new, it is not defined in many printed outlets, but a definition has been provided by Dictionary.com (2009).

“A carbon footprint is the sum total of carbon dioxide and other greenhouse gasses emitted into the atmosphere, either directly or indirectly, by a person, group, product, or process.”

A major contributor of carbon emissions is the burning of fossil fuels. Their usage can be reduced when local foods are brought into the kitchen, because the food does not have to be transported by truck to reach the consumer. It should be noted,

that this reduction in fossil fuels required to move food from the field to the market can be offset by inefficient distribution methods such as consumers driving to multiple venues to purchase food. Distribution points can reduce some of these inefficiencies. Farmers markets and specialty food shops are examples of distribution points.

Home preparation of food can save money and permit the consumer to have more control over food quality and diversity. This lifestyle is becoming a greater trend with 24-35 year olds as more popular culture celebrities espouse the benefits of reducing environmental impact while enjoying the social aspect of farmers markets and food preparation at home (Mintel Oxygen, 2009). Buying locally grown in-season foods may also reduce the strain on a household's food budget especially during economically difficult times and when consumers are seeking organic alternatives to conventionally grown foods.

Certified organic foods sold in the U.S. have exhibited a dramatic increase. As demand for these products has increased, so has their availability. Now, some mainstream supermarkets offer certified organic products, specified organic sections, and/or store-branded organic product lines. Organic products generally have a premium price, and that can leave some consumers turning to farm direct purchasing to save money.

Farm direct sale occurs when consumers purchase food products directly from farms. These transactions take place as people acquire food from farms, roadside stands, farmers markets, and specialty shops that dedicate shelf space to local products. Many people also view these gathering places as social or family events.

The increase in the farmers markets across the country has been substantial. In 1994, there were 1,755 registered markets, by August, 2008 there were 4,685 markets (AMS, 2008). As farmers markets grow in number and size, they also grow in diversity. Some now offer arts, crafts, clothing, fresh flowers, nursery stock, and prepared foods in addition to fresh produce and animal products. These events can be important to residents of urban areas who desire a greater connection to the land, their community, and the production of their food.

To facilitate this desire, there is a federal program called “community supported agriculture” (CSA). The idea originated in Japan and Switzerland during the 1960’s as a partnership between farmers and consumers who were all seeking market stability. The program moved into the U.S. in the 1980’s and has seen slow, but steady escalation (NAL, 2009). The basic principle of this program guides consumers to pledge, or prepay for an allotment, to the farm before the growing season starts. In doing so, they share some of the risk during the growing season, connect with the land and seasons, and can benefit from a good harvest year by receiving more food at the pledged price. The repayment during harvest is a certain allotment of in season foods. The CSA program offers many positive attributes to farmers as well. Farmers are able to use prepaid dues for growing expenses as they arise, have stable market prices, and have a reduced need for marketing during harvest seasons. CSA programs are increasing across the U.S. In 1993 there were over 400 registered farms participating in the program, and by 2007 that number climbed to 12,549. Over 300 of the CSA participating farms are found in Oregon (NASS, 2008).

In Western Oregon, the 300 miles previously referred to is a geographic distance that permits the generation of a wide variety of products and foods. Locavores try to take advantage of the diversity of the area by purchasing a majority of household foods from this radius. A radius of this size, centering on Corvallis, Oregon, would allow locavores to purchase products originating from distinct geographical regions of the Pacific Northwest. Seafood is harvested from the Pacific Ocean. Types of nuts, fruits, vegetables, and domestic animal products are grown in the Willamette Valley. Dryland crops of grains, fruits, vegetables, legumes, forages, seed stock, and grazing animals are produced in Southern and Eastern Oregon. Domestic animal products produced in large quantities within Oregon include milk, cheese, eggs, beef, lamb, and poultry.

Use of federal grazing permits, pasturing sheep on grass seed fields, and the ability to grow large amounts of forage enable some Oregon farmers and ranchers to produce animals that consume diets consisting mainly of local ingredients. This is not necessarily the situation when considering producers involved with poultry.

Poultry are monogastric animals and must consume a more nutrient dense diet of concentrates as they do not have a rumen to produce energy. Most poultry diets consist of primarily corn and soybean meal because these ingredients are some of the most economical components which closely match their nutritional needs. In 2008, feed corn grown in Eastern Oregon, approximated 185,000 tons (NASS, 2009). Soybeans are not grown in the Western United States in any reportable amount. The absence of soybean production in and near Oregon results in the importation of

soybean meal from the Midwest and Eastern sections of the U.S. For small scale poultry producers who choose to grow and market locally produced animals, fed locally produced feeds, other ingredient options must be explored.

Legumes for Sustainability

There are many alternative protein sources that can be grown in Oregon, but few offer the benefits of legume grains. While soy is a legume, it is not efficiently grown in Oregon. Legume grains are the seeds produced by legume plants. Three primary benefits of growing legumes are, the grains have high concentrations of crude protein, can be used for human or animal consumption, and the plants are capable of nitrogen fixation reducing the need for exogenous plant fertilizer. The concentration of crude protein found in legume grains such as dry beans or peas can be considerably higher than those found in other commercial grains such as corn or wheat. Higher protein concentrations can be useful when formulating diets where protein requirements range between 19% and 23%. Grain legumes can help bridge the gap between corn (9% protein) and the higher protein requirements of poultry. The benefit of human and animal consumption of grain legumes is two-fold. When commodity prices are high, growers can target sales of some legume grains toward human food use, and cull grains can be used by feed processors. During times of low economic value, or when other commodities are too costly, legume grains can be directed into the animal feed market if processors know how to properly handle and include them. The last major benefit of growing legumes is nitrogen fixation. Nitrogen fixation is

the ability to glean atmospheric nitrogen (N_2) and convert it into ammonia (NH_3) which can be used for plant growth. This process is the result of a symbiotic relationship with *Rhizobium* bacteria. The bacteria exist in root nodules on the legume plant, and with the assistance of photosynthetic energy, convert sufficient nitrogen for plant growth. Depending on soil quality, many areas of the U.S. can grow legumes successfully without the addition of nitrogen fertilizer, and others may use little. When the seed pod (or the entire plant) is removed from the soil, the fixed nitrogen is removed also (Lindermann and Glover, 2003). In instances when the annual crop is tilled into the soil for fertilizer, the nitrogen within the plant is added back to the soil. This process can be an integral part of crop rotation used in sustainable agricultural practices.

Sustainable agriculture describes the integration of three concepts: improving and maintaining environmental health, promoting economic farm profitability, and enhancing the social and economic equity of owners and laborers (Feenstra, 1997). The overall goal of sustainable agriculture is to meet the needs of current consumers without impairing the capability of future generations to meet their own needs. According to these goals, sustainable agriculture and expansive plantings of a single crop (commercial monoculture plantations) are not sustainable because they deplete the top soil of specific nutrients and require application of synthetic fertilizer and pesticides, both of which are in direct conflict with sustainability. Improper or inefficient application of fertilizer can result in the contamination of groundwater and surrounding areas with unused compounds. Integration of legumes and multiple

species into a monoculture system can reduce environmental impacts by reducing the dependence on fertilizer for nitrogen and chemical pesticides, herbicides, and fungicides to combat diseases associated with non-rotated monoculture crops (Van Bruggen, 1995).

Growing grain legumes in a sustainable agricultural system offer many benefits to both the grower and the environment - a source of green manure, low input crop production, and a viable feedstuff for humans and animals. These factors make grain legumes an interesting and potentially valuable feed ingredient to investigate.

CHAPTER 2

LITERATURE REVIEW

Phaseolus Vulgaris

Phaseolus vulgaris, the common bean, has been used throughout history to feed both humans and animals. Though raw beans have a relatively low concentration of digestible protein (Koehler, et al., 1986), they are an important crop in areas with limited access to animal proteins or technological advances for further food processing. Significant research has been conducted in areas around Israel and Cameroon/Nigeria where agriculturalists are trying to cultivate broiler populations. Bean research has focused on the growth and performance of common varieties for use in broiler feeds because of the limited, or nonexistent, need for synthetic nitrogen fertilizer to ensure adequate production. Nitrogen fixation and drought resistance are important environmental adaptations of legumes, because it allows growers to produce a useful food and feedstuff in poor soils and climates not favored by the growth of many other high-protein plants.

Today, there are many different cultivars available, each with unique properties (Wychoff, et al., 1983). Some of the variable characteristics are environmental adaptability, color, size, texture, palatability, and antinutritional factors. These qualities help establish a distinct nutritional profile for each variety. For some of the most common varieties: white, navy, red kidney, pink, red Mexican, and pinto types, there are great differences, not only overall concentrations of crude protein, but

also amino acid profiles and the relative nutritional value of the protein (Koehler et al., 1987).

When including beans in the broiler diet, the bird's age can affect how well the chicks utilize the ingredient. Farrell, et al., (1999) reported that broilers aged 22-42 days were better able to utilize variety of leguminous grains in their diets than chicks fed the same proportions during the first 21 days of life. Evidence of this phenomenon is supported by Fualefac Defang, et al. (2008) who demonstrated that when *Phaseolus* is used in growing broiler diets, heavier final bodyweights were obtained in all test diets when a commercial broiler starter diet was fed during the four-week starter phase.

Pisum sativum

Pisum sativum, the field pea, is another leguminous animal feedstuff gaining popularity. Commercially blended samples of field peas, averaging 23% protein and 46% starch, have a good balance of crude protein and metabolizable energy for human and animal consumption (Cowieson, et al., 2003). Field peas have high concentrations of lysine averaging 1.67%, but relatively low contents of methionine and cystine (Hickling, 2003). High concentrations of lysine, in excess of NRC requirements, have been reported by Kerr, et al., (1999) to significantly improve overall performance and breast meat yields in male broilers. When compared to some cereal grains, field peas have a relatively low oil content with an ether extract of 1.4%; however, the available

oil is similar in molecular structure to that of cereal grains in that it is primarily polyunsaturated (Hickling, 2003).

Field pea varieties have variable nutrient and antinutritional factors similar to those found in *Phaseolus* (Gabriel, et al., 2008). Igbasan and Guenter (1996a) reported that a 50% inclusion of peas significantly reduced bioavailable energy (AME), apparent protein digestibility (APD), and starch digestibility. Yellow- and green-seeded cultivars had similar AME and starch digestibilities, while both AME and starch digestibility were significantly lower in the brown-seeded samples. Significant APD variation exists among pea cultivars. Kluth, et al. (2005) reported that the digestible energy of diets containing 30% field peas were significantly lower than the (iso-energetically balanced) corn-soy control diet which contained no pea meal.

Other differences among pea cultivars were noted by Vidal-Valverde, et al., (2003) who evaluated 18 pea cultivars for concentrations of nutritional compounds and antinutritional factors. Of the cultivars analyzed, there were general trends between the gross appearance of peas and some nutritional and antinutritional values. Seed coat color serves a role in categorizing the analyzed values. Trypsin inhibitor activity is highest in peas with yellow seedcoats. Peas with brown seedcoats had the highest concentration of phytate, but were lowest in verbascose and sucrose sugars. Light green seedcoats had the highest lysine values and dark green varieties the richest thiamine and riboflavin concentrations. The size was also an indicator of nutritional value as smaller peas had increased concentrations of protein nitrogen, thiamine,

riboflavin, verbascose, and phytate. Medium sized peas had the lowest values for verbascose, alpha-galactoside, and riboflavin. The largest peas were characterized by the lowest concentrations of inositol pentaphosphate.

The inclusion of peas into the rations of broilers has been well studied and there is a general consensus for optimal inclusion of between 10% and 30% depending on the balance between diminished growth capacity and the current feed costs (Farhoomand and Saed Poure, 2006; Cowieson, et al., 2003; Hickling, 2003, McNeill and MacLeod, 2001; Farrell, et al., 1999; Perez-Maldonado, et al., 1999). When peas are included at 10% of the diet broilers demonstrate a slight, but significant, decrease in feed intake. A significant decrease in feed intake occurred when broilers were fed pea meal from days 1-42 at a rate of 20% of the total ration. Though feed intake was reduced, feed conversion from days 0 to 42 was minimally affected (McNeill and MacLeod, 2001). The proposed reason for this was a demonstrated preference by chicks against rations containing pea meal. Bernard and MacLeod (2001) reported that between days 7 and 14, when broiler chicks were offered a choice between field pea meal and a control diet of wheat and soy, chicks selected the control diet 1.8-fold over the pea meal diet. To support good production from layers, a maximum inclusion of 25% peas was recommended to the egg industry (Perez-Maldonado, et. al, 1999). Farrell, et al., (1999) made a broiler industry recommendation for the optimal inclusion of peas to be 30% of the diet.

Effects of Legumes on Carcass Quality

Feeding field peas and common beans to commercial poultry does not appear to affect meat quality. McNeill, et al. (2004) used a triangle type test where panelists were offered cooked breast meat samples from birds fed rations formulated with 0%, 10%, or 20% pea meal. This style of taste test is proctored so birds fed two different diets are compared. Taste panelists could not significantly distinguish between samples of cooked breast meat from broilers fed any of the three diets tested. Similar results were obtained when feeding peas to market turkeys. Savage, et al. (1986) reported feeding ground yellow peas had no detrimental affects on the sensory evaluation or meat quality of market turkeys. Similar promising results have been found when feeding *Phaseolus* to broilers. When broiler diets containing 45% field beans, were fed for the first nine weeks of life, no differences in flavor were detected by a taste panel, and only slight differences were recorded by gas chromatographic analysis (Grey, et al., 1972). Marzo, et al. (2007) fed 10% extruded kidney bean meal to broilers and reported no significant differences in muscle composition or metabolism.

Antinutritional Factors

An obstacle preventing widespread use of *Phaseolus vulgaris*, and to a lesser degree, *Pisum sativum*, is the presence of antinutritional factors. According to Liener (1989), the negative constituents of beans are protease inhibitors, lectins, tannins, amylase inhibitors, phytic acid, and oxalates. Plants can accumulate antinutritional

substances in a variety of tissues, though the seed is a common repository. Substantial evidence suggests the functions of these compounds are to provide defenses against insects, as many compounds found to be antinutritional for humans and livestock are also toxic or growth inhibitors for certain insects as well (Murdock and Shade, 2002). While some of the aforementioned bean characteristics are toxic, some are not, but still must be neutralized. “Non-toxic,” though still unfavorable, characteristics can elicit unfavorable biological responses from animals, and unless degraded, can reduce the nutritional facility of legume products (Liener, 1979).

Protease Inhibitors

Protease inhibitors, specifically inhibitors of trypsin and chymotrypsin, prevent those enzymes from performing a crucial role in the digestion of proteins. Early work in this area of nutritional physiology established that the protease inhibitors did not prevent pancreatic secretions, but neutralized the pancreatic enzyme itself causing additional trypsin secretions from the pancreas (Lyman and Lepkovsky, 1957). By 1962, Liener reported that one of the principle physiological responses to trypsin inhibitors was pancreatic hypertrophy. The mechanism for growth inhibition (reported in multiple species) was not well understood; however, dietary supplementation of methionine was recommended for proper development when diets included ingredients containing protease inhibitors.

Liener (1989) discovered the action of trypsin inhibitors in the small intestine. As trypsin inhibitors enter the gut they reduce free trypsin. This reduction initiates

cholecystokinin release from the intestinal mucosa. Cholecystokinin, a hormone, incites pancreatic acinar cells to supply more trypsin, chymotrypsin, amylase, and elastase, which have high concentrations of sulfur-containing amino acids. The inactivation of trypsin causes a negative feedback loop, and when continued, can result in the loss of important sulfur-containing amino acids. This promotes hypertrophy and hyperplasia of the pancreas and an overall growth depression.

Clarke and Wiseman (2001) bolstered previous findings that pancreatic enlargement in broiler chicks can be associated with dietary trypsin inhibitor activity (TIA). Their work suggests a linear correlation between TIA intake (ranging between 14.8 and 1.9 mg/g of sample) and the pancreas to bodyweight ratio. Their study also demonstrated that when TIA was reduced, from 4.5 mg/g to 1.9 mg/g, there was a considerable improvement in the apparent digestibility of all 16 amino acids measured. Al-Marzooqi and Wiseman (2002) used near-isogenic pea lines to reveal an inverse linear correlation between enhanced ileal digestibility of amino acids, especially methionine, and reduced dietary TIA in broiler chicks. In 2005, Clarke and Wiseman reported that coefficients of digestibility for separate amino acids varied considerably between the full fat soybeans and soybean meals used to feed broiler chicks, but did not correlate with TIA concentrations. This indicated that there are other factors found in soybean meal and full fat soybeans that affect the digestibility of amino acids.

Trypsin inhibitor activity has also been shown to confound the digestibility of the amino acid dimer, cystine. Kakade et al. (1969) demonstrated that a

disproportionate amount of cystine found in navy beans was contained within the trypsin inhibitor isolate. Unless the navy beans were heat treated (the proven method at that time), the cystine would be measurable, but not available, for chick growth. Much of the research performed on trypsin inhibitors has used soybean samples. Soybeans have been used because they are ubiquitous in the manufacture of human food and animal feedstuffs and are of great economic value. Inhibitors found in other legumes cause pancreatic hypertrophy and depress growth similar to inhibitors that have been studied in soybeans (Liener and Kakade, 1980).

Lectins

Lectins are non-immunoglobulin proteins, usually in the form of glycoproteins, which specifically recognize and reversibly bind to complex carbohydrate moieties without altering their covalent structure (Pusztai, 1991). Recent advances in structural analysis and molecular cloning have allowed for further partitioning of the plant lectin group and one of the four major families is the legume lectins (Van Damme, et al., 1998). Leguminous lectins are the best characterized because they are found in many common foods and act as antinutritional factors. According to Sharon and Lis (1990), lectins have different specificities for carbohydrates, with very similar physiochemical properties. Lectins found in legumes are distinctive in that they contain tightly bound Ca^{2+} and Mn^{2+} ions associated with highly conserved amino acid residues associated with carbohydrate binding. They are usually comprised of two or four subunits and each subunit has a single site to bind one carbohydrate.

Plant lectins serve two major biological roles, protein storage and protection (Van Damme, et al., 1998). When lectins are found in high concentrations in seeds and tissues they represent protein storage which can be mobilized for plant growth and development. This storage, which is part of the reproductive process, doubles as a plant protection mechanism when herbivorous organisms attempt to consume components vital to plant survival and reproduction.

Murdock and Shade (2002) suggest three likely interactions for lectin disruption of feeding, health, and digestion in insects. First, plant lectins can bind with the carbohydrate moieties associated with insect membranes associated with chemosensory and food detection and identification. Lectin binding can secure the protein receptors intended to gather the chemical signals of food. The second possible site of disruption is the peritrophic matrix of the insect midgut. The peritrophic matrix is a protective layer of proteins, glycoproteins, chitin, and glycosaminoglycans secreted by the epidermal cells of the midgut. Lectins can bind with the thin protective layer and disrupt the matrix causing perforations and allowing bacteria to invade the delicate surface of the digestive epithelium. The last possible site of lectin action is direct binding with digestive epithelial cells in the midgut of the insect. This binding interferes with normal digestion and absorption as the digestive epithelial cells are responsible for the release of food degrading enzymes and absorption of digested chemicals. Any of these disruptions, or their concerted efforts, would hinder growth, development, and the reproductive capacity of predatory insects.

Considering the effects lectins can have on insects, it is no surprise that they can also retard growth and development in higher animals. In a review of lectins by Huisman and Tolman (1992), the principle effect of lectins in higher animals was binding to the intestinal wall mucosa. This action results in cellular damage, decreased absorption, a change in enzymatic activity, and loss of endogenous protein due to the detachment of damaged epithelials. These effects can result in reduced weight gain and inefficient feed conversion through decreased nutrient digestibility and nitrogen retention.

For many years, it has been known that raw *Phaseolus vulgaris*, especially the red kidney bean variety, is unsuccessfully tolerated by animals fed diets primarily consisting of these beans. Some animals have died within a week or two of continuous consumption. This situation prompted Liener (2002) and his team to develop a system for the mass-production of purified kidney bean lectin which proved to be a very powerful growth inhibitor of rats and chicks. Later studies used this purified kidney bean lectin to help better understand its structure and function in the digestive tract.

Purified phytohemagglutinin (PHL) lectins isolated from red kidney beans were fed to weanling rats to determine growth, absorptive, and bacterial overgrowth maladies in the small intestine. The PHL was found to hinder growth rates and promote characteristic changes of the microbial ecology (the nature and distribution of bacteria and protozoa at the mucosal lining) reducing absorption of nutrients (Banwell et al., 1985). While previous findings have shown that the effects of lectins cannot be

extrapolated from one species to another, other studies have demonstrated the deleterious effects of *Phaseolus vulgaris* on poultry (Van der Poel, et al., 1990; Wagh, et al., 1963; and Emiola, et al., 2007a).

Tannins

Tannins are water soluble polyphenolic compounds that can form complexes with proteins and carbohydrates. Once tannin-protein or tannin-carbohydrate complexes are formed, they become insoluble enzyme-resistant compounds (Deshpande, et al., 1986). The biological effects of tannins occur, in part, from their ability to bind with proteins, which happens more easily than with carbohydrates. The affinity for proteins to bind with tannins is due to the hydrogen bonds of the tannin interacting with the carboxyl oxygen of the peptide group on proteins, thus, bonding ability differs greatly with the composition of the proteins. Compounds having the strongest affinity to bind with tannins are comprised of a large open structure with high concentrations of proline and no bound carbohydrates (Butler, et al., 1984).

The concentration of tannins varies among legume species and also among varieties within species. There are no apparent associations between the total tannins and the seed yield or seed protein content, although environmental differences can affect tannin levels, the cultivar contributed more to the overall phenolic concentration (Wang et al., 1998). There is a correlation between concentrations of tannins and the color of the seedcoat its pigment intensity. The general trend is for seeds with more intensely colored seedcoats and flowers to have higher concentrations of condensed

tannins. Similar correlations have been noted in common beans (Caldas and Blair, 2009), and field peas (Igbasab and Guenter, 1996a). Griffiths and Mosely (1980) demonstrated the colored-flowering bean plants with higher polyphenolic contents reduced trypsin and alpha-amylase activity in the intestines of rats and also induced more enzymatic secretion from the pancreas. These occurrences were not observed for white-flowering bean plants which had low polyphenolic contents.

The negative association of growth in monogastrics and tannins has long been known. In 1964, Chang and Fuller reported the growth depression in chicks fed sorghum tannins. Further studies reinforced the link between tannins and growth retardation, but offered suggestions to alleviate the effects of tannins in feed. While Fuller et al. (1967) noted that growth depression increased with greater inclusion of tannic acid, they also discovered that supplemental methionine, arginine, and choline improved chick growth rate and detoxified the tannic acid. At a 1% dietary inclusion of tannic acid the supplemental amino acids reduced the negative effects, while at a 0.5% dietary inclusion of tannic acid, the three amino acids completely eliminated the deleterious effects. Later, the metabolic fate of dietary tannins was described by Potter and Fuller (1968). Tannic acid fed to hens was hydrolyzed to gallic acid which was in large part O-methylated and excreted in the uric acid as 4-O-methyl gallic acid. This explains why supplementing high-tannin diets with methionine and choline had beneficial effects on chick growth.

Protein is not the only dietary constituent whose digestion and absorption are adversely affected by dietary tannins. An interaction between polyphenols and

carbohydrates had been proposed as an explanation for the difference between the calories lost in undigested protein in high-tannin and low-tannin diets fed to rats (Featherstone and Rogler, 1975). Deshpande and Salunkhe (1982) pursued this concept and presented their in vitro study of the interaction of tannic acid with legume starches. They found that bound tannic acid decreased the digestibility of all six of the legume starches tested; azuki red beans, bush beans, split yellow peas, small red beans, and California kidney beans.

As with most antinutritional factors, species react differently to tannins. This general idea was supported by Huisman et. al (1990) who fed raw beans to three commonly studied monogastrics; pigs, rats, and chicks. The live weight of piglets fed raw beans was drastically reduced, while live weights in the rats and chicks were unaffected. Addition of dietary casein did not improve piglet weight gain. This indicated that the growth depression was not due to an amino acid deficiency, but because of other toxic effects.

Piglets are usually more sensitive than chicks, when fed tannins, and chicks are more sensitive to tannin concentrations than rats. Amino acid supplementation did not alleviate growth depression in piglets, but was beneficial for chicks as they used methyl groups from methionine (and choline) to detoxify the tannins. Rats were relatively unharmed by the addition of high-tannin feedstuffs to the diet. Rats when fed high-tannin feedstuffs; however did experience parotid gland hypertrophy and produced a group of salivary proteins with an exceptionally strong affinity for tannins. This group of proteins contained more than 40% proline and lacked sulfur-containing,

and aromatic, amino acids. This may be a protective metabolic adaptation that binds tannins at first contact with the digestive tract and thereby reducing its deleterious effects.

Amylase Inhibitors

Amylase is an enzyme found throughout the digestive tracts is used to digest starch in many animal and insect species. The proposed plant function of this enzyme was to protect the plant and its reproductive elements from the digestive processes of insects and other predatory species. Amylase inhibitors impede the digestion of starch in animals by binding with pancreatic amylase and forming insoluble moieties leaving the starch without the digestive enzyme (Savelkoul, et al., 1992).

In kidney beans, an alpha-amylase inhibitor has been found to inhibit the digestion of starch (Jaffe and Vega Lette, 1968). Marshall and Lauda (1975) discovered and named this alpha-amylase inhibitor found in kidney beans, as phaseolamin. They reported that it is specific to animal alpha-amylase and ineffective toward enzymes in the corresponding plant, bacteria, or fungi. Phaseolamin had no activity toward any other hydrolytic enzymes tested.

The degree to which amylase is inhibited is dependent on many factors, including environmental pH and temperature, time of interaction, prior hydrothermic treatment, and concentration of the inhibitor (Jaffe and Vegga Lette, 1968; Thompson, 1993; and Champ, 2002). Competitive binding of starches should also be considered when discussing alpha-amylase. While tannic acid and catechin do not inhibit alpha-

amylase they are able to bind with some starches and fortify them against the digestive enzyme (Deshpande and Salunkhe, 1982).

A red kidney bean inhibitor has been shown to have minimal effects on some species and no effects in others, so it seems to be of minor antinutritional importance (Huisman and Tolman, 1992)

Phytates

Phytate is the salt of myo-inositol hexaphosphate, also known as phytic acid or IP6, the form in which many plants store phosphorus (Eeckhout and De Paepe, 1994). Monogastric animals can make little use of this form of phosphorus because they have negligible endogenous phytase activity, the enzyme responsible for separating phosphorus from the phytate complex through hydrolysis (Pallauf and Rimbach, 1997).

The structure of phytic acid lends itself to relatively high reactivity. The inositol ring has six phosphate groups attached, and each phosphate group has two protons. This leaves phytic acid with twelve dissociable protons which can easily bond with available cations. Phytic acid readily chelates with divalent and trivalent metal ions including calcium, copper, iron, zinc, and magnesium and even some proteins (Urbano, et al., 2000). The complex formed when phytic acid binds metallic ions reduces their absorbability in the intestinal tract and can lead to malnutrition if the diet is not supplemented with additional nutrients (Liener, 1979). Cowieson, et al., (2006) stated that not only does the phosphate complexation of minerals reduce

mineral availability, but also has other effects on growth as it inhibits mineral participation as cofactors in enzymatic activity. This is especially important in marginally sufficient diets where certain minerals may already be in short supply.

Two mechanisms are used to hydrolyze phytate to inositol and free phosphates - non enzymatic cleavage, and phytase activity. Phytases are ubiquitous throughout communities of micro-organisms, plants, and animals (Urbano, et al., 2000), though minimally expressed in some monogastrics. The micro-organisms of the rumen account for the ability of ruminants to utilize phytate bound phosphorus. They are endowed with microbial phytase and hydrolyze the phytate before it reaches the point of absorption in the ruminant digestive process (Pallauf and Rimbach, 1997). Poultry cannot employ this system and must rely on exogenous phytase. Phytase enzymes act on phytic acid by hydrolytic catalysis which dephosphorylates the compound, thereby reducing the chelating ability of the phytate (Urbano, et al., 2000).

Non-enzymatic hydrolysis of phytates can occur through soaking, autoclaving, toasting, and fermentation. Great Northern, kidney, and pinto beans were processed through various methods of soaking and heating and phytate concentrations were measured. Phytic acid had the greatest degradation from soaking in distilled water (eighteen hours) and/or conventional cooking for 90 minutes (Vishalakshi, et al., 1980). When fermentation for the purpose of reducing phytic acid was evaluated by Chitra, et al. (1996), phytic acid concentrations dropped between 26 % and 50 % depending on the variety of legume undergoing fermentation.

Phytic acid obstructs the normal use of the basic protein residues. Liener (1989) proposes that this obstruction is the reason why phytate inhibits digestive enzymes such as alpha-amylase, pepsin, and pancreatin. When exogenous phytases are supplied in poultry rations containing high concentrations of phosphates, birds demonstrate an improvement in weight gain, mineral retention, metabolizable energy, and amino acid digestibility (Cowieson and Adeola, 2005). The mechanism responsible for improvements in amino acid utilization is likely mediated through reduced endogenous losses of amino acids and greater retention of dietary amino acids (Cowieson, et al., 2004).

The addition of phytase to poultry diets can markedly reduce the amount of phosphorus required in the ration. In the absence of phytase, nonphytate-bound phosphorus was required at higher concentrations to optimize tibia ash, bodyweight gain, and feed conversion rate. In the presence of 800 units of phytase per kilogram of feed, reduced nonphytate phosphorus input was required to maintain the same measurement standards. Assessment of fecal samples determined that lower concentrations of phosphorus were excreted from the phytase-supplemented group (Yan, et. al, 2001). Cowieson, et al., (2006) demonstrated that in addition to hydrolyzing phytates, the phytase enzyme can partially ameliorate the negative effects phytates have on protein utilization, therefore, causing a reduction in the unused portions of phosphorus and nitrogen that end up in the excreta. Financial expenditure may have prohibited some from using exogenous phytases in feeds as dicalcium phosphate is relatively inexpensive, but as environmental concern over the

concentration of nitrogen and phosphorus in animal wastes increases, supplementation may become more cost effective than recovery methods.

There are a variety of factors that can influence the use of phytate phosphorus (and calcium) by broilers. Some confounding, or enhancing, factors of utilization are: microbial phytase, cholecalciferol, and the ratio of calcium to total phosphorus in the broiler diet (Sebastian, et al., 1998). Supplemented phytase linearly increased bodyweight gain, feed intake, toe ash, and calcium and phosphorus retention. Those same measurements were synergistically improved by the addition of cholecalciferol. All measurements were reduced by the widening ratio of calcium to total phosphorus in both the presence and absence of supplemental cholecalciferol and phytase (Qian, et al., 1997).

A study designed to determine the amount of calcium that is absorbed from common beans indicated that some measured calcium was bound and unavailable for absorption. To evaluate the relation of phytate to reduced absorption, phytase was added to some of the samples. While fractional absorption rose, it was not to the predicted level. The authors suggested that this was probably due to the high concentration of oxalate which can act in a similar fashion (Weaver, et. al, 1993).

Non-starch Polysaccharides and Oligosaccharides

Non-starch polysaccharides (NSP) are complex carbohydrates composed of water soluble hemicelluloses, pectic substances, beta-glucans, gums, and mucilages, and water insoluble cellulose (Prosky and DeVries, 1992). Fructans, glucomannans,

and galactomannans are a small group of NSP that serve the plants as storage for polysaccharides. Legume grains have relatively high concentrations of NSP and can impair normal growth and development in poultry by resisting endogenous enzymatic degradation in the digestive tract (Classen and Bedford, 1991).

Polymers of glucose and pentose sugars (glucans and pentosans, respectively), mucilages, and pectins are gummy constituents of NSP which promote increased water intake, endogenous water secretion into the alimentary tract, and ingesta viscosity. Increased gut viscosity causes feed to remain in the small intestine longer and form a bolus which can physically inhibit enzymatic attack. Lengthening passage time may increase the growth of microbial populations and allow the gummy glucans and pentosans to form a partial barrier over the glycocalyx of the microvilli, the main absorption point in the intestinal lumen. The partial barrier excludes larger molecules from passing through the lumen during absorption. This prohibition has the greatest effects on the uptake of fats, cholesterol, and fat soluble vitamins, as they must bind with large bile salt micelles for transport. If NSP are not controlled in the broiler diet they can result in a specific fat malabsorption syndrome, fecal loss of vitamins and minerals, and wet/sticky feces (Anderson and Chen, 1979; Classen and Bedford, 1991; Bedford, 1996; Dingle and Wiryawan, 1997; and Cheek, 1998).

Oligosaccharides are carbohydrates that, when hydrolyzed, will produce three to nine monosaccharides. Huisman and Tolman (1992) provide a review of the complications that can arise when feeding high concentrations of oligosaccharides. Some oligosaccharides bypass digestion in the small intestine due to a lack of

appropriate enzymes. Once in the large intestine they are degraded by bacterial alpha-1,6-galactosidase and the sugar monomers are fermented into carbon dioxide, hydrogen, and methane along with the volatile fatty acids: acetate, propionate, and butyrate. The products of bacterial fermentation can cause diarrhea for the birds consuming large amounts of these unprocessed polysaccharides. The results of continuous consumption can be reduced feed intake due to discomfort, wet droppings, and damp litter. Common beans are often associated with oligosaccharides. In the human nutrition literature this group of sugars is often under the heading of “flatulence factors” because of the fermentation products produced in monogastrics. The most common oligosaccharide in pinto, Great Northern, and kidney beans is stachyose from the raffinose family of oligosaccharides (Vishalakshi, et al., 1980).

Alleviation of Antinutritional Factors

When the factors discussed above are degraded leguminous nutrients are better utilized. Many techniques have been reported to partially or fully ameliorate some of the antinutritional factors found in grain legumes. Some of the most common processing techniques including: soaking and aqueous heating, autoclaving, dry toasting, dehulling, dietary supplementation, germination, and extrusion.

Soaking and Aqueous Heating

Soaking legume seeds diminishes the effects of some antinutritional factors as leaching of those factors occurs. Tegui and Fon Fru (2007) fed broilers three

differently-treated diets containing a 15% inclusion of *Phaseolus vulgaris* that had been soaked in water (12 hours). Significantly improved bodyweight gains and feed conversions were observed in broilers fed either the 0% bean inclusion control diet or soaked and extruded beans (145C), than with either soaked and heated (dry oven 100C for 15 minutes) or soaking alone.

Heating legume seeds while soaking can hasten the process of leaching and produce better results than soaking alone. Using both peas and beans, it was determined that aqueous heating at 95C for 30 minutes decreased the association between phenolic compounds and starches (Deshpande and Salunkhe, 1982). Ofongo and Ologhobo (2007) reported that processed kidney beans can be used for 50% protein for protein replacement of soybean meal or ground nut cake in broiler diets. Cooking kidney beans (one hour in water) had better results than soaking (12 hours) followed by decortication. The best performance was obtained by decortication followed by cooking, as many antinutritional factors are localized in the seedcoat.

Autoclaving

Autoclaving is the process of quickly raising the temperature of seeds using heat and moisture without the leaching (loss of nutrients transferring from the seeds to the water) which occurs during boiling. Goatcher and McGinnis, 1972 demonstrated that autoclaving *Phaseolus* improved chick growth. Four varieties of beans were fed to growing chicks at approximately 17% of the total ration. Raw beans caused

significant pancreatic enlargement which autoclaving reduced, but did not completely eliminate.

When yellow-seeded peas were included at 20% in the rations of broiler chicks from days 7-42, autoclaving (120C for 20 minutes in a layer 20 mm thick), and aqueous cooking (100C for 20 minutes after soaking 12 hours) significantly improved bodyweight gain and feed conversion rate compared to raw peas (Farhoomand and Saed Poure, 2006). Peas autoclaved for six and a half hours at 60C exhibited a 25% loss of phytate due to hydrolysis from newly activated phytase (Beal and Mehta, 1984).

Autoclaving has been found to increase the growth potential of multiple species fed diets with legume constituents. Researchers investigated improvement of the nutritional value of common beans by autoclaving. Six cultivars were tested and larval growth of the red flower beetle was used as a measurement of nutritional value. Samples were autoclaved at 112C for 30 minutes. Autoclaved beans significantly improved larval growth and decreased larval mortality versus raw bean diets fed to other groups; however, both performances did not compare with the control diet (Wyckoff, et al., 1983). Van der Poel (1990) found that of all heat treatment parameters investigated, autoclaving beans at 119C for five to 10 minutes was optimal when feeding beans to piglets. Lectins were degraded without destroying the majority of available lysine, an indicator of excessive protein damage.

Dry Toasting

Dry toasting is the process of dry heating seeds either in an oven or over an open fire. Van der Poel, et al. (1990) reported a 14-day feeding trial in chicks which determined the best growth rates occurred when common beans were dry toasted for at least 20 minutes at 105C. Dry toasting for longer periods of time did not result in further growth improvement.

Researchers tested the trypsin inhibitor activity in soybeans that had been dry toasted in a toasting oven type commonly available on small family farms. Kricka, et al. (2003) subjected soybean samples to various toasting times, temperatures, and thicknesses. The optimum cooking process for reducing trypsin inhibitor activity in soybeans was determined to be 125C for 15 minutes layered in a thickness of no more than 30 mm per tray. This particular processing method was described as specific for monogastric diets.

Though improvement of legumes for use as a monogastric feedstuff is possible, some still do not promote growth as well as diets containing soybean meal. Pone and Fomunyan (2004) roasted kidney beans in a 20 cm layer for 30 minutes when the temperature reached 100C at the center of the tray. When 20% of the diet was toasted kidney beans, experimental birds had poorer performance than birds fed soybean meal as the main protein source. This demonstrated that kidney beans cannot replace the inclusion of soybeans without affecting bird performance.

Dehulling

Dehulling is the process of removing the outer fibrous seedcoat. The value of this process is documented when feeding common beans, but is debatable for peas.

Dehulling peas had little or no effect on the apparent metabolizable energy-nitrogen adjusted, apparent protein digestibility, and starch digestibility when only considering yellow- and green-coated varieties; however, a significant improvement was noted after dehulling brown-seeded peas (Igbasan and Guenter, 1996b). Farhoomand and Saed Poure (2006) found that the dehulling process significantly improved the bodyweight gain of broiler chicks fed a 20% inclusion of yellow peas between the ages of 7 and 21 days. It is unclear if this is an age dependent interaction or if this procedure requires further evaluation.

Supplementation

Dietary supplementation with exogenous enzymes, carbohydrases, and essential amino acids may help alleviate some of the negative effects of feeding leguminous seeds. Igbasan and Guenter (1996b) demonstrated that broilers can consume 20% of their diets as peas without any performance reduction. They further concluded that a ration including 40% peas can provide satisfactory results if it is mixed in a diet that also includes the supplementation of crude protein and essential amino acids in an excess of 15% of the NRC requirements for broiler growth.

Supplementing bean diets with methionine proved beneficial when feeding autoclaved beans. Improvements of growth and feed conversion efficiency varied

depending on bean variety. Addition of methionine to rations containing uncooked beans had no effect, nor did supplementation with threonine, tyrosine, and valine (Goatcher and McGinnis, 1972). Cowieson, et al. (2003) documented that between days 1-21, broilers fed rations with 30% peas demonstrated reduced weight gain, nutrient digestibility, and feed conversion. These effects were partially reduced by supplementation with dietary enzymes (which positively affected the control as well) and carbohydrases. In the presence of such feed additives, the authors proposed that some pea cultivars have substantial promise as vegetable protein sources for growing chicks.

Germination

Mikola (1983) offers an overview of the proteolysis during germination using three distinct stages. First, the hydrolysis of the seed invokes the liberation of amino acids which form enzymes responsible for converting reserve constituents into transportable and usable substances. Then, hydrolysis continues and reserve proteins are degraded down to amino acids which are then used for plant growth. Finally, the bulk of the cellular proteins and enzymes are mobilized for seedling development prior to the beginning of autotrophic growth where exogenous energy sources are required to sustain plant growth.

Germination and fermentation significantly improved legume quality. Germination reduced phytic acid contents and total dietary fiber concentrations, and increased the degree of in vitro protein digestibility (Chitra, et al., 1996). Peas

germinated for ten days demonstrated increased phytase activity by twelve-fold and reduced the phytate concentration by 75 percent (Beal and Mehta, 1984).

Extrusion

Extrusion is the process of forcing a substance through a die, or series of dies, under extreme pressure and heat. This process is commonly used to produce a variety of foods and pelletized feeds. Arija, et al. (2006) found that processing kidney beans via extrusion significantly improved weight gain, feed consumption, and feed conversion (when included in the diet at 10%, 20%, and 30%) over raw kidney beans added at the same inclusion rates. Though performance was improved by extrusion, the kidney bean meal did not meet the performance level of the control corn/soy diet.

Comparison of Methods

Some researchers have compared various methods for processing legumes to improve their quality and determine the most useful processing methods. Emiola, et.al., (2007a) reported their findings using an approximately 25% inclusion of kidney beans in broiler diets. The aqueous heated and dry heated diets did not hinder growth or organ development when compared to the control (0% bean inclusion) diet, and they had significantly better performance than the raw and raw-dehulled rations. Birds performed slightly better on the aqueous heated diets than the dry heated versions.

Emiola, et.al. (2007b) replaced 50% of the dietary soybean meal with kidney bean meal treated by one of three methods: aqueous heating (kidney beans were added

to 100C water for one hour and oven dried at 85C for 48 hours), dry toasting (a thin layer of seeds were oven heated at 120C, stirring occasionally until the beans turned from white to golden- about 20-25 minutes), and dehulling. Average daily feed intakes were similar for all treatments, but average daily gains and feed conversion efficiencies were higher in the aqueous heat treated beans than either dry toasting or dehulling.

Many of the procedures performed to ameliorate the effects of antinutritional factors found in legume grains have been studied with respect to human nutrition and may not be feasible in underdeveloped areas, or locally for small feedmills or farmers constructing their own rations (Van der Poel, 1990). Access to specialized equipment can be a limiting factor for the utilization of much of the current information available on further processing of legume grains for monogastric nutrition. The heat used during the extruding and pelletizing processes has proven useful in degrading certain antinutritional constituents in feed ingredients, but not helpful to those who do not have access to the required equipment.

In consideration of this deficit, the feed processing techniques and rearing environments used in the following experiments were developed to mirror conditions that can be found at grow sites where producers cannot depend on the environmental consistency provided by insulated buildings and thermo-regulated ventilation. These realizations are the basis for the procedures used throughout the feed processing and environmental design phases of the experiments described below.

CHAPTER 3

METHODS AND MATERIALS

This study was completed in three phases. The first and third phases of research were conducted using the same set of twelve pens. The second phase took place in a single room of a different building on the same farm.

Experiment One

Birds

Cobb x Cobb day-old broiler chicks (N=327) were obtained from a commercial source. The chicks were randomly assigned to one of twelve pens. Each treatment group consisted of 27 chicks and each control group consisted of 28 chicks.

Environmental Design

The research facility was an uninsulated building with cement flooring and cinder block walls to the external environment. This facility was chosen to simulate brooding conditions commonly found in small flock rearing situations. The twelve pens each measured 4.88 m. long by 2.44 m. wide. Environmental consistency was achieved through an open-air design using propane brooders placed approximately 1.2 m. above the wood shavings used for litter and spaced such that two pens shared the output of one heater. The purpose of the propane brooder was to temper the air of the enclosure against December weather conditions. In addition to the generalized heat provided by the brooders, each pen had one 250 watt infrared bulb for a point source heat. The outer areas of the pens averaged 18.3C, while directly under the infrared

bulbs was consistently 54.5C. The extreme variation in temperature allowed chicks to self-regulate for thermal comfort as feed and water sources were positioned along the temperature gradient. At 26 days of age, the propane brooders were disconnected and the infrared light bulbs were the only supplemental heat source. Fluorescent ambient lighting was provided 24 hours per day.

The linear pen arrangements lead to variation in water pressure, water quality, and proximity to the 12th pen which had an additional external cinder block wall. Recognition of these possibly confounding factors prompted a block randomization of treatments. Blocks were designated as follows: block 1, pens 1 to 4, pens 5 to 8 were block 2, and pens 9 to 12 were block three. Every block contained one replicate of each of the four treatments.

Treatments

Chicks were fed experimental and control mash feeds and water ad libitum throughout the duration of the experiment. All feed was weighed and recorded prior to distribution to each pen. The four treatment diets were as follows: corn-soybean control, 30% pinto beans, 30% Great Northern white beans, and 30% small red beans, see Table 1 for diet formulations.

Measurements and Samples

Prior the start of this experiment the three bean varieties were analyzed for their nutrient components (Table 2) and amino acid composition (Table 3). The trial began on day one and continued until the chicks reached 42 days old. During this time, and immediately after, measured and observational data were documented. On

days 7, 21, and 42, individual bird weights were recorded. Final bodyweights were subjected to an analysis of variance (ANOVA) testing and the treatments were compared using the Tukey-Kramer test. Feed conversion rates were calculated and compared on a per pen basis using Duncan's Multiple Range test. Different tests for statistical significance were used based on software availability. At the conclusion of the trial, four birds (two males and two females) were randomly selected from each pen for analysis. The accumulations of abdominal fat pads were noted in addition to any other anatomical inconsistencies. Tissues for histological samples were collected on day 42. Samples were removed from the crop, duodenal loop - including the pancreas (2 cm. from the distal end), and 2 cm. from the colon/cecal junction. Samples were excised and placed in 10% buffered formalin for histological analysis.

Experiment Two

Birds

Cobb x Cobb day-old broiler chicks (N=144) were obtained from the same commercial source as in experiment 1. The chicks were randomly assigned to one of 24 cages. Each cage contained six birds at the beginning of the trial.

Environmental Design

Experiment two was conducted in one environmentally controlled room. A total of 24 cages were used for the experiment. Each cage measured 40.6 cm. wide and 50.8 cm. deep. Attached to the front of the cage was a feeding tray which spanned the cage width. One, height-adjustable, nipple drinker was allotted per cage. To

maintain proper temperature, twelve 250 watt infrared heat lamps were placed above the line of cages wherein two cages across from each other benefited from the same lamp. Fan ventilation was used to maintain air quality and temperature.

Treatments

Chicks were allowed specified feeds and water ad libitum throughout the duration of the experiment. All feed was weighed and recorded prior to distribution to each pen. The six treatment diets were as follows: 30% raw pinto, 30% dry toasted pinto, 30% raw Great Northern white beans, 30% dry toasted Great Northern white beans, 30% raw small red beans, and 30% dry toasted small red beans (Table 1). These diets were mixed in the same fashion as in the first experiment with the exception of dry toasting the flour prior to the inclusion into the heat-treated diets. The dry toasting process consisted of a two part procedure. First, each variety of bean was ground to the same particle size as other constituents of the diet. Then the resulting bean flour was placed onto metal trays in a 3 cm. layer and oven heated at 130C for 35 minutes. The treatments were block randomized and four replicates of each treatment were tested. Each block was designated as two cages (back to back) by three cages long.

Measurements and Samples

The trial was conducted from day one until the chicks reached 21 days old. During this time, and immediately after, measured and observational data were recorded. Individual bird weights and feed consumption were recorded on day twenty-one. Final bodyweights were subjected to an analysis of variance (ANOVA)

testing and the treatments were compared using the Tukey-Kramer test. During the trial, observational data was recorded focusing on leg health and manure accumulation near the vents, as these issues arose during experiment one. With the first presentation of pasty vents, scores were taken on a per cage basis. Feed consumption was recorded throughout the trial and conversion rates were determined on a per cage basis at the conclusion of the trial. Feed conversion rates were compared using Duncan's Multiple Range test. One bird was randomly selected from each cage for necropsy and general assessment.

Experiment Three

Birds

Cobb x Cobb day-old broiler chicks (N=300) were obtained from the same commercial hatchery as the previous experiments. The chicks were randomly assigned to one of twelve pens. Each treatment replicate consisted of 25 chicks.

Environmental Design

This trial was conducted in the same system as experiment one with one modification; the use of an additional 250 watt infrared heat lamp in each pen. One replicate of each treatment was assigned to each designated block as was described in experiment one.

Treatments

Chicks were allowed access to the approximately isonutritional experimental and control mash diets and water ad libitum throughout the experiment. All feed was

weighed and recorded prior to distribution to each pen. The four treatment diets were as follows: corn-soybean meal control, 30% peas, 76% peas (no soybean meal or soy oil), and 20% ground peas in addition to 10% sprouted peas (20/10). The ingredients and formulation ratios are found in Table 1. The treatment groups that were assigned the 20/10 diet received the 30% pea diet until day 25 of the trial. Then, the groups were fed a diet containing 30% peas, 20% of the peas were mixed into the mash diet and 10% were measured out daily, sprouted, and fed as a top dressing over the mash feed. To accurately determine 10% of the diet, daily consumption values were recorded from the treatment groups which were assigned a constant 30% pea diet. The average daily intake was determined on a per bird basis, multiplied by 0.10, and applied to each 20/10 treatment group based on the number of birds in each pen. After 10% of the diet was calculated, the proper amount of dry whole peas could be measured and entered into the sprouting process.

Sprouting Process

The sprouting process took place over four days. On day 0, the amount of whole dry peas was weighed according to the procedure described above and put into a plastic tote filled with water. The water was drained from the tote and the contents were agitated on day one. On day 2, the peas were again, agitated and covered with water. The contents of the tote were agitated and drained on day three. On day four, the sprouted peas were ground and fed out to the 20/10 treatment groups. Sprouted peas were ground in a food processor for three seconds which split and quartered each pea, but did not result in homogeneous mixture. The grinding process was required as

the sprouted pea was far larger, 2.8-fold increase in mass, than the dried pea, and could not easily be consumed whole by the chicks.

Measurements and Samples

Prior to the beginning of this experiment the two types of peas used for ration mixing and sprouting were analyzed for the basic components important to poultry nutrition (Table 4). The trial began on day one and continued until the chicks were 42 days old. During this time, and immediately after, measured and observational data were documented. On days 7, 21, and 42, individual bird weights were recorded. Final bodyweights were subjected to an analysis of variance (ANOVA) testing and the treatments were compared using the Tukey-Kramer test. Feed conversion rates were calculated and compared on a per pen basis using Duncan's Multiple Range test. Different tests for statistical significance were used based on software availability. Two birds from each pen were taken for further examination. The accumulations of abdominal fat pads were noted in addition to any other anatomical irregularities.

CHAPTER 4 RESULTS AND DISCUSSION

Experiment One

Final bodyweights were subjected to an analysis of variance (ANOVA) testing and the treatments were compared using the Tukey-Kramer test. Feed conversion rates were compared on a per pen basis using Duncan's Multiple Range test. Some interactions of minor importance were investigated, but found to be insignificant. Block designation, sex dispersion among treatments, and sex/treatment interactions were not significant ($P > .05$).

Broiler chicks fed a ration containing 30% raw *Phaseolus vulgaris* (pinto, Great Northern white, and small red) had a significantly ($P < .05$) reduced bodyweight (Table 5). Pinto beans outperformed ($P < .05$) Great Northern white beans and small red beans with a mean bodyweight of 1.15 kg., versus 1.21 kg. and 1.19, respectively. There was no statistical difference between the mean bodyweights of birds fed Great Northern white beans and small red beans. Similar bodyweight depression in broilers was also seen by Pone and Fomunyan (2004) and Arija et al. (2006).

Feed conversion rates followed the same relationship wherein all diets containing *Phaseolus* demonstrated lower performance when compared to the corn-soy control diet (Table 6). The control diet had a conversion value of 1.49. This ratio was significantly ($P < .05$) better than any of the diets incorporating beans. Pinto beans (2.25) outperformed ($P < .05$) Great Northern white beans (2.44) and small red beans (2.66), between which there was no statistical difference.

Upon the necropsy of two males and two females from each of the twelve replicates, no gross internal abnormalities and abdominal fat pad accumulation. No obvious lesions or malformations were noted in any treatment group. Control treatment groups had well developed fat pads as typically found in commercial broiler birds. Broilers fed pinto beans had very thin and sparse deposits of fat over the abdominal area. No visible fat accumulations were found in the abdominal regions of any birds fed white or red beans.

Histological examination of tissue samples from three most probable regions of the digestive tract to be affected by the inclusion of *Phaseolus* in chick diet revealed little damage in most birds (Heidel, 2009). Birds fed the control and pinto diets lacked any lesions in the crop. Some crop samples from chickens fed white (1 out of 3) and red beans (2 out of 3) had a modest inflammation of submucosal layer and were likely only an incidental finding. Two out of three duodenum/pancreas samples taken from all four treatments had modest, but well defined, lymphocyte aggregates in the pancreatic connective tissue, but not the pancreas itself. No acinar or islet damage was associated with this inflammation. No significant lesions were found in the colon/ceca samples taken from birds of any treatment group.

Observations were recorded throughout the experiment. During the second week, frequent instances of manure accumulation near the birds' vents (pasty vents) were seen in both the pinto bean and Great Northern white bean treatments. Pasty vents were noted only toward the end of the third week in the small red bean treatment groups, accompanied by extreme size variation among the birds not noticed in other

groups. Between days 21 and 42, there were substantial numbers of control treatment birds showing signs of splay leg or other conditions of lameness. Conversely, no more than two birds from any replicate of other treatments had such symptoms.

Physical conditions of the pens were considered when designing this experiment as certain blocks within the experimental system may have an environmental advantage over other areas. This was accounted for in the design and tested for significance at the completion of the experiment. Block assignments had no significant impact on various pen replicates within the same type of treatment.

Two statistical anomalies were found at the conclusion of this experiment, but both are believed to be artifacts of maturation rate. When the interaction of sex and treatment was tested for significance, the data were suggestive, but not conclusive ($P = .07$), that the treatments affected the sexes differently. This can be explained by understanding that the control birds were far larger (almost two-fold) than the birds in other treatment groups. As control birds were better able to reach their genetic potential through more suitable nutrition, males were able to differentiate from, and become larger than females. This differentiation is common and usually seen in broilers after the third week of life. Pinto bean fed birds had a slightly greater separation between the weights of males and females than those treated with Great Northern white and small red beans, but substantially less than control-treated broilers.

The other interesting statistic involved the random assignment of day-old chicks to treatment groups. The chicks arrived in straight run boxes which were randomly dispersed to each replicate pen. The sex of the individual bird was not

determined until day 42 of the experiment (Table 4). Sex was determined using the secondary sex characteristic of comb development. The percent of finished male broilers should be approximately 50% considering the sample size of 327 individuals, but the actual number was less. For the control, pinto, white, and red diets the percents male were, 45%, 42%, 37%, and 35%, respectively. This follows the same general pattern of final bodyweights and may be an artifact of delayed maturation brought on by a less adequate level of nutrition.

Of the three experimental treatments, raw pinto beans appear to be the most promising for common usage in small flock systems. While the feed conversion and growth rates were not comparable to the control diet (sufficient for conventional commercial production), they may be suitable for small flock producers with different requirements. In the six week growing period, birds fed a diet with 30% pinto beans required 1.5 times the amount of feed to produce a unit of bird than control broilers. In addition to less efficient feed utilization, in the six weeks allotted for this experiment pinto bean-treated birds only grew to 66% of their control counterparts' potential.

There are rare situations where these results would be acceptable, but for those situations the use of pinto beans may be justified. When the price of pinto bean culls is low enough and other feed ingredients are unobtainable, (physically or cost-wise) their inclusion may be worthwhile. Other factors that may be important to the feasibility of using pinto beans surround the situation of the local grower. If there is a specialty market that is accepting of smaller birds or has a preference for birds with a

reduced subcutaneous fat covering, the premium price may offset low bodyweights and suboptimal feed conversion. It should also be considered that if the farm overhead is inexpensive, allowing the birds a longer growing period may enhance overall performance. Additional research is required to address whether different growing periods (longer than 42 days) and environmental situations (free range, pastured, multi-species, large flock, small flock, etc.) can affect the utilization of raw beans.

Experiment Two

Broiler chicks fed from days 1 through 21 on diets which included 30% heat-treated (H-T), specifically dry toasting, *Phaseolus vulgaris* showed some improvement in growth depending on the variety of bean (Table 7). The mean bodyweights for chicks consuming raw and H-T pinto beans, 0.44 and 0.46 kg., respectively, were not different. Birds fed H-T Great Northern white beans had significantly ($P < .05$) higher bodyweights than birds fed the raw Great Northern white beans 0.42 vs. 0.34 kg., respectively. Heat treatment of white beans improved weight gain to the level achieved by chicks in the pinto bean groups. There was no difference between the raw (0.32 kg.) and H-T (0.39 kg.) small red bean treatments, only suggestive evidence ($P = .06$). There was no significant ($P < .05$) difference among H-T red (0.39 kg.), H-T white (0.42 kg.), and raw pinto beans (0.44 kg.). This result supports Van der Poel, et al. (1990) who reported that dry toasting common beans improved chick performance between days one and twenty-one.

These results suggest that heat-treatment affects varieties of *Phaseolus vulgaris* in distinct ways. While all varieties demonstrated higher bodyweights after dry toasting, only white beans had a statistically significant difference. When considering the findings from the first experiment, where pinto beans outperformed both white and red beans (between which there was no difference), it is understandable that the improvement found in white beans divided the nutritional abilities of H-T and raw white beans. H-T white beans promoted chick growth similar to that of the pinto bean treatments and the raw white beans were similar to the small red bean treatments.

Dry toasting beans (ground into flour) prior to inclusion into broiler diets significantly ($P < .05$) improved feed efficiency of chicks fed Great Northern white beans (2.51 vs. 2.94) and small red beans (2.60 vs. 3.10) over raw bean flour. There was no difference in the mean conversion rates of chicks consuming H-T pinto beans (2.38) versus raw pinto beans (2.36). Conversion rates (Table 8) should only represent a relationship between these varieties of beans and not true conversion rates, as cage rearing allowed birds to dig through feeders and waste feed. Substantial accumulations of feed were found on the floor throughout the trial. While the amount of rejected feed appeared consistent among all cages, it was not recoverable for accounting practices.

The heat treatment of the *Phaseolus vulgaris* varieties examined in this experiment did not affect the incidence of pasted vents or leg quality. Pasty vents were first noted on day six. Beginning with the first presentation of this condition, the incidence of pasty vents and the degree of fecal accumulation were recorded on a per

bird basis every three days until the end of the experiment. At the conclusion of this trial, an average of three out of the six birds in each cage had some degree of manure accumulation. The rate of occurrence was consistent between heat-treated and raw versions of all bean varieties; although, chicks treated with pinto beans presented the earliest during the trial. Manure accumulation varied between a thin non-viscous layer and a hardened protruding buildup. Again, there was no correlation between the severity of the pasty vents and raw or H-T treatments. Leg health was monitored throughout the experiment, and no incidence was observed. It was expected that lameness might occur as some chicks in the first trial experienced leg health issues and birds grown in cages tend to have increased lameness. The absence of lameness may be due to the short duration time of the experiment.

Two birds from each of the 24 cages were examined during necropsy. No gross anatomical irregularities were noted in any group.

Heat-treatment of beans acts differently on different cultivars (Wyckoff et al., 1983), and heat treating certain types of beans can improve overall broiler performance. Results of this experiment provide evidence in support of this trend. Pinto beans are unaffected by the method of heat-treatment performed in this experiment. Great Northern white beans are significantly better at supporting chick growth and efficient feed utilization after dry toasting. Small red beans promote better feed efficiency after heat-treatment and there is suggestive, but inconclusive, evidence that dry toasting may improve bodyweight gains in chicks between days one and twenty-one.

If this method of heat treatment can improve the nutritional value of Great Northern white beans and small red beans to the level demonstrated by raw pinto beans, it would be a reasonable assumption that the feasibility of including them into poultry diets should also be similar to that of raw pinto beans. If cull white and red beans are available locally, less expensive than other protein sources, the grower has a niche market accepting of a smaller carcass or is able to let the birds grow longer, and has access to a facility for this method of heat-treatment, then they may be an economically useful option. While agriculturalists are known for creativity and adaptability, there are many caveats to the successful use of these feedstuffs which require a very specific set of circumstances in order to capitalize on their use. More research should be conducted to determine if the effects of dry toasting are different when birds are grown beyond 21 days, as birds react differently to legume grains after 21 days of growth (Farrell, et al., 1999; Bedford, 1996).

Experiment Three

The mean bodyweight (2.54 kg.) of broilers fed a ration containing 30% *Pisum sativum* was not different ($P < .05$) than the mean bodyweight (2.66 kg.) of birds consuming the control formulation (Table 9). These results support the findings of Igbanan and Guenter (1996b) who found no reduction when pea meal was included at 20% of the total ration. Results from this experiment are not in agreement with Cowieson et al. (2003) who reported 30% inclusion of pea meal into wheat/soybean meal diets reduced broiler bodyweights from days 1 through 21; however, experiment

three ran twice as long. The mean bodyweight (2.39 kg.) of birds started on the 30% field pea diet and transitioned at day 25 to the 20/10 sprouted pea diet was not different ($P < .05$) than birds fed the 30% field pea ration throughout the 42 day experiment. Broilers treated with the 76% pea diet (no soy products) had a mean bodyweight of 2.06 kg. which was significantly ($P < .05$) lower than the means produced by birds fed the other treatments examined during this trial.

The feed conversions for this experiment are found in Table 10. All three experimental treatments had significantly ($P < .05$) different rates of feed conversion. The 76% pea formulation had the highest ratio of feed conversion (1.79), followed by the 30% pea diet (1.71), and then the sprouted 20/10 ration (1.52). The rate of feed efficiency for the sprouted 20/10 ration was the highest of all of the experimental treatments and not significantly ($P < .05$) different than the control diet (1.45). The ratio of abdominal fat pad accumulation to body size appeared to be consistent throughout all treatment groups in this experiment.

Birds offered sprouted grains did not achieve any higher bodyweights than the group treated with 30% dried peas; however, feed conversion was better. It is unclear if this is a result of better feed utilization alone, or if it is a statistical artifact of the birds being smaller which can increase feed efficiency. Birds offered sprouted grains averaged 0.27 kg. less than the control birds, a difference of about 10 percent. Conversely, chicks raised on the 76% pea diet were approximately 23% smaller than the control birds, but had the lowest level of feed efficiency of all four treatments tested.

While some small flock producers and pastured poultry enthusiasts claim birds perform better when fed a “living” food, overall weights were unaffected. The information behind these claims focuses on the change, or turnover, of amino acids during the germination process. It has been demonstrated that the amino acid profile is altered by sprouting (Jones and Tsai, 1976; Chen and Thacker, 1978; Mikola, 1983), but whether this change can positively affect poultry growth is unknown. Birds in the 20/10 treatment group were allowed access to sprouted feed for 17 days, and it is possible that more time is needed to demonstrate an improvement in performance. This treatment may have a better impact on birds fed sprouted grains for a longer period of time, perhaps in a layer system as opposed to a short-term broiler system.

Field peas have proven successful in broiler diets at the 30% inclusion level. This knowledge is useful to both the commercial and small flock producer as no further accommodations are required to implement their use. Because field peas can be grown in the Pacific Northwest and do not retard growth at lower inclusion levels, *Pisum sativum* was considered as a possible replacement for soybean meal in the 76% pea “no soy” diet.

The two main considerations for the removal of soy products in this ration formulation are based on consumer concerns. The first concern addressed by this diet was local production of human food. Some consumers are interested in not only where their food is produced, but also what the animals have been fed and where that feed was grown. This highlights the issue that there is a growing awareness of the carbon footprint left by consumers, and a desire to support one’s local economy.

Since soybeans in the United States are primarily grown in the Midwest and perform poorly in Oregon and Washington, considerable fuel use is required to move soy products and byproducts to the West Coast. This expenditure of fossil fuel adds to the amount of carbon gasses released into the atmosphere. When ration constituents are produced closer to the point of utilization carbon emissions can be minimized. The second concern, whether legitimate or perceived, surrounds the health implications of feeding soybean meal. A small segment of consumers want to avoid foods which contain soybean products. The reasoning behind this desire is to avoid the ingestion of phytoestrogens which may alter serum estrogen concentrations, and hexane used during oil extraction and GMOs which may have unknown implications. A myriad of publications from both popular media and scholarly outlets continue to debate whether the concentration of phytoestrogens found in soybeans have any affect on individuals consuming soy. Participants in this debate also have differing views on whether any possible effects on health are positive or negative. For consumers skeptical of GMOs, it can be very difficult to find non-GMO soybeans as 91% of the soybeans planted in the U.S. in 2009 were genetically engineered (ERS, 2009b). These concerns have typically been voiced with regard to soy products intended for human consumption, but a subgroup of consumers is beginning to raise the same concerns for the health of the animals they wish to consume.

Conclusion

The use of *Phaseolus vulgaris* in broiler diets at 30%, can inhibit growth versus a more common corn-soy protein based ration. Performance is significantly enhanced when the bean flour is dry toasted in a layer 3 cm. deep at 130° C for 35 minutes. Pinto beans are the exception, as chicks showed no growth improvement after the heat treatment of pinto beans. Even after heat treatment, broilers do not grow as well when beans are included in the diet at this rate; therefore, inclusion rates should be relatively low. The use of first run, or cull beans, may be financially beneficial depending on the prices of commodities, local availability, access to equipment for dry toasting, and the desire of regional specialty markets.

When *Pisum sativum* was included at 30% of the total diet no significant reduction in growth was noted. This concentration appears to offer appropriate support for growth and is recommended for all growers who can financially justify the use of peas in their formulations. Sprouting peas did not enhance broiler performance and is not recommended for a 42 day growing period. Broilers fed a diet of 76% peas, and which included no soy products, demonstrated the worst performance. The final bodyweights were the lowest and feed conversion rates were the highest of all diets tested in this experiment. The average bodyweight was 0.6 kg. less than the control diet. The use of this type of diet should be limited to growers with specific needs such as meeting a niche market desires for broilers raised exclusively on local products.

Common beans and field peas can be grown in both Oregon and Washington. Having local access to legume grains may allow small producers and commodity

purchasers for custom feedmills the ability to buffer themselves against fluctuating commodity prices. When larger mills are able to control costs by securing vast quantities of protein sources, small scale buyers may be able to reduce their costs by using alternative feedstuffs, such as legume grains, in the formulation of custom diets. In addition to the general economic buffering, custom producers may be able to capitalize on specialty markets demanding genetically modified organism-free (GMO-free) feedstuffs. This concept arrives at an important time when there is a growing concern in certain consumer populations and it is becoming more difficult to find soybean meal and feed corn that has not been genetically modified to enhance plant growth and hardiness.

Table 1:
Ration Formulations of All Diets Tested in Experiments 1, 2, and 3.

	Control [#]	PB [#]	WB [#]	RB [#]	30% Pea [#]	20/10 Pea [#]	76% NS [#]
Pinto beans	-	30.0	-	-	-	-	-
G. N. White Beans	-	-	30.0	-	-	-	-
Small Red Beans	-	-	-	30.0	-	-	-
Dry Peas	-	-	-	-	30.0	20.0	76.0
Sprouted Peas	-	-	-	-	-	10.0	-
Corn	52.9	32.0	29.4	36.0	33.9	33.9	7.5
Soybean Meal (48%)	38.5	29.4	32.0	25.4	27.5	27.5	-
Corn Gluten Meal	-	-	-	-	-	-	7
Soybean Oil	4.0	4.0	4.0	4.0	4.0	4.0	-
Corn Oil	-	-	-	-	-	-	4.9
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Dical. Phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Sodium Chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Premix *	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix ^	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calc. Protein (%)	23.36	23.62	23.65	23.36	23.34	23.56	23.38
Calc. Energy (Mcal/g)	3.06	2.95	2.94	2.99	2.95	3.00	2.92

[#] Control-corn and soy based commercial style diet, PB-30% pinto bean diet, WB-30% Great Northern white bean diet, RB-30% small red bean diet, 30% Pea-30% pea diet, 20/10 Pea-20% dry peas in the mash with 10% of the ration top dressed as sprouted peas diet, 76% NS- 76% peas with no use of soy products diet.

* Vitamin premix supplied per kg. of diet: calcium, 964 mg.; iron 1.50 mg.; vitamin A, 8,079 I.U.; vitamin D3, 3,029 I.U.; vitamin E, 30 I.U.; menadione, 2.02 mg.; riboflavin, 5.05 mg.; d pantothenic acid, 12.12 mg.; niacin 50.49 mg.; vitamin B₁₂, 0.01 mg.; folic acid, 1.26 mg.; thiamine, 2.02 mg.; pyridoxine, 4.04 mg.; biotin, 0.15 mg.

^ Mineral premix supplied per kg. of diet: calcium, 228 mg.; copper, 6.05 mg.; iodine, 1.01 mg.; iron, 30.23 mg.; manganese 60.46 mg.; selenium, 0.15-0.18 mg.; zinc, 60.46 mg.

Table 2.
Phaseolus vulgaris Nutritional Analysis (% DM)

	Pinto Bean	White Bean	Red Bean
Crude Protein	22.00	19.00	27.20
Calcium	0.40	0.20	0.12
Phosphorus	0.29	0.49	0.63
Sodium	0.00	0.00	0.00
(Dry Matter)	90.40	89.50	89.20

Table 3.
Phaseolus vulgaris Amino Acid Analysis (%)

	Pinto Beans	White Beans	Red Beans
Alanine	0.80	1.00	1.01
Arginine	1.53	1.75	1.91
Aspartic acid	2.18	2.80	2.94
Glutamic Acid	2.60	3.34	3.50
Glycine	0.79	0.95	0.96
Histidine	0.55	0.65	0.70
Isoleucine	0.87	1.06	1.15
Leucine	1.49	1.90	2.03
Lysine	1.31	1.60	1.69
Methionine	0.17	0.21	0.21
Phenylalanine	1.03	1.34	1.44
Proline	0.78	0.96	0.95
Serine	1.02	1.37	1.43
Threonine	0.80	1.12	1.10
Tyrosine	0.65	0.77	0.84
Valine	0.98	1.27	1.30
TOTAL	17.55	22.09	23.16

Table 4.
Pisum sativum Nutritional Analysis (% DM)

	Feed Peas*	Sprout Peas^
Crude Protein	26.70	24.10
Calcium	0.12	0.08
Phosphorus	0.50	0.42
(Dry Matter)	90.30	88.40

*Feed Peas are a commercial blend of field peas used in a the production of the mash diets

^Sprout Peas were the peas used for the sprouting experiment. These peas were used in place of the feed peas because the seedcoats were not scarred to prevent sprouting.

Table 5.

Experiment 1: Final Bodyweights (kg) of Broiler Chicks Fed Common Beans on Day 42 Expressed as Replicate and Overall Means.

<u>Treatment</u>	<u>Rep. 1</u>	<u>Rep. 2</u>	<u>Rep. 3</u>	<u>X ± SE</u>	<u>Male</u>
Control	2.43	2.28	2.17	2.29 ± .049 ^a	45%
30% Pinto	1.46	1.60	1.48	1.51 ± .053 ^b	42%
30% G.N. White	1.30	1.16	1.18	1.21 ± .051 ^c	37%
30% Small Red	1.30	1.10	1.14	1.19 ± .051 ^c	35%

^{abc} Treatment means with different superscripts are significantly different (P < .05).

Table 6.

Experiment 1: Mean Feed Conversions of Broiler Chicks Fed Varieties of Common Beans. Days 0 Through 42.

<u>Treatment</u>	<u>Rep. 1</u>	<u>Rep. 2</u>	<u>Rep. 3</u>	<u>Mean</u>
Control	1.44	1.51	1.53	1.49 ^a
30% Pinto	2.34	2.12	2.33	2.25 ^b
30% G.N. White	2.48	2.19	2.71	2.44 ^c
30% Small Red	2.33	2.95	2.80	2.66 ^c

^{abc} Treatment means with different superscripts are significantly different (P < .05).

Table 7.

Experiment 2: Final Bodyweights (kg) of Broiler Chicks Fed Heat-Treated (HT) and Raw Common Beans on Day 21 Expressed as Replicate and Overall Means.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	X \pm SE
HT Pinto	0.45	0.47	0.46	0.45	0.46 \pm .013 ^a
Raw Pinto	0.42	0.44	0.43	0.48	0.44 \pm .013 ^{ab}
HT White	0.44	0.45	0.30	0.41	0.42 \pm .013 ^{abc}
Raw White	0.36	0.30	0.35	0.32	0.34 \pm .013 ^d
HT Red	0.36	0.43	0.40	0.37	0.39 \pm .013 ^{bcd}
Raw Red	0.34	0.29	0.32	0.32	0.32 \pm .013 ^d

^{abcd} Treatment means with different superscripts are significantly different ($P < .05$).

Table 8.

Experiment 2: Mean Feed Conversions of Broiler Chicks Fed Varieties of Common Beans Heat-Treated (HT) and Raw. Days 0 Through 21.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean
HT Pinto	2.74	2.19	2.32	2.33	2.38 ^a
Raw Pinto	2.48	2.34	2.46	2.17	2.36 ^a
HT White	2.58	2.18	3.03	2.39	2.51 ^a
Raw White	2.70	3.49	2.75	2.94	2.94 ^{bc}
HT Red	2.66	2.64	2.48	2.62	2.60 ^{ab}
Raw Red	3.21	3.38	2.86	3.00	3.10 ^c

^{abc} Treatment means with different superscripts are significantly different ($P < .05$).

Table 9.

Experiment 3: Final Bodyweights (kg) of Broiler Chicks Fed Diets Utilizing Field Peas on Day 42 Expressed as Replicate and Overall Means.

Treatments	Rep. 1	Rep. 2	Rep. 3	X + SE	Male
Control	2.61	2.80	2.61	$2.68 \pm .055^a$	56%
30% Pea	2.55	2.53	2.53	$2.53 \pm .055^{ab}$	48%
20/10 Pea	2.32	2.34	2.38	$2.37 \pm .054^b$	42%
Peas/No Soy	1.98	2.21	1.94	$2.04 \pm .055^c$	44%

^{abc} Treatment means with different superscripts are significantly different ($P < .05$).

Table 10.

Experiment 1: Mean Feed Conversions of Broiler Chicks Fed Diets Utilizing Field Peas. Day 0-42.

	Rep. 1	Rep. 2	Rep. 3	Mean
Control	1.43	1.45	1.48	1.45^a
30% Pea	1.66	1.75	1.73	1.71^b
20/10 Pea	1.50	1.50	1.54	1.52^a
Peas/No Soy	1.82	1.79	1.77	1.79^c

^{abc} Treatment means with different superscripts are significantly different ($P < .05$).

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