Evaluating Canola (*Brassica napus*) Meal and Juncea (*Brassica juncea*) Meal With or Without Supplemental Enzymes for Two Commercial Strains of Laying Hens

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

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DALHOUSIE UNIVERSITY

FACULTY OF AGRICULTURE

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Abstract

Two trials compared the effects of canola meal (CM) and juncea meal (JM) with and without dietary enzyme supplementation, on production performance, egg quality, bone quality and liver health characteristics of white- (WSLH) and brown-shell egg laying hens (BSLH). A total of 360 Lohmann LSL-Lite White (Trial 1, WSLH) and 300 Lohmann Brown-Lite (Trial 2, BSLH) laying hens were fed one of 10 isoenergetic and isonitrogenous diets (Soybean meal, 10 % CM, 20 % CM, 10 % JM or 20 % JM with or without a dietary enzyme cocktail of Superzyme OM™ and Bio-Phytase™) for 48 weeks. Based on the results of production performance, incidence of mortality, egg quality, bone quality, and liver health data, up to 20 % CM or JM can be included in diets of WSLH and BSLH without detrimental effects. Enzyme should be included in diets for both WSLH and BSLH.

List of Abbreviations and Symbols Used

Analysis of Variance ………………………………………………………..ANOVA
Bone Breaking Strength …………………………………………………………BBS
Bone Mineral Content ………………………………………………………..BMC
Bone Mineral Density ………………………………………………………..BMD
Brown-Shell Egg Laying Hen ……………………………………………………BSLH
Calcium ……………………………………………………………………………Ca
Calcium Ion ……………………………………………………………………...Ca^{2+}
Canola Meal ………………………………………………………………………CM
Centimeter …………………………………………………………………………cm
Concentration ………………………………………………………………………[ ]
Conjugated Linoleic Acid ………………………………………………………CLA
Dry Matter …………………………………………………………………………..DM
Erucic Acid …………………………………………………………………………..EA
Extracellular Matrix ………………………………………………………………ECM
Fatty Acid ……………………………………………………………………………FA
Fatty Liver Hemorrhagic Syndrome ……………………………………………...FLHS
Feed Consumption ………………………………………………………………...FC
Feed Conversion Ratio ……………………………………………………………..FCR
Gastrointestinal ……………………………………………………………………GI
Glucosinolate ……………………………………………………………………….GLS
Gram …………………………………………………………………………………...g
Haugh Units …………………………………………………………………………HU
Hepatosomatic Index ………………………………………………………………..HSI
High Erucic Acid Oil ………………………………………………………………HEA
Juncea Meal ………………………………………………………………………….JM
Kilogram ……………………………………………………………………………..kg
Litre …………………………………………………………………………………L
Low Density Lipoprotein ………………………………………………………….LDL
Low Erucic Acid Oil ……………………………………………………………….LEA
Metabolisable Energy ……………………………………………………………….ME

Micromole ……………………………………………………………………………μmol
Miligram ………………………………………………………………………………..mg
Millimeter ……………………………………………………………………………...mm
Millimole ……………………………………………………………………………mmol
Newtons …………………………………………………………………………………N
Non-Phytate Phosphorus ……………………………………………………………...NPP
Non-Starch Polysaccharide ……………………………………………………………NSP
Parathyroid Hormone …………………………………………………………………PTH
Percent …………………………………………………………………………………...%
Phosphorus ……………………………………………………………………………..P
Pre-Press Solvent Extraction ………………………………………………………...PPSE
Quantitative Computed Tomography ………………………………………………...QCT
Rapeseed Meal …………………………………………………………………….RSM
Shell Breaking Strength ……………………………………………………………….SBS
Soybean Meal …………………………………………………………………….....SBM
Standard Error of the Mean …………………………………………………………..SEM
Specific Gravity ……………………………………………………………………...SG
Vitamin D …………………………………………………………………………..Vit D
White-Shell Egg Laing Hen ………………………………………………………..WSLH
With Enzyme ………………………………………………………………………...+ E
Without Enzyme ……………………………………………………………………..– E

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Chapter 1. Introduction

Poultry diets in Canada typically consist of corn or wheat and soybean meal (SBM), and/or canola meal (CM), depending on the region of the country where the diet is formulated. With an increased interest in converting corn to ethanol for biofuel production, the cost of corn is expected to rise, leaving producers to find a more economical source of energy and protein for their livestock.

In Canada, canola is mostly grown in the Prairie Provinces, although there is an ever increasing amount of canola being grown in rotation in the east. New varieties have been developed that have lower glucosinolates (GLS; 10.55 μmol/g in the non-toasted meal) (Newkirk et al. 2003b) and lower tannin levels than previous cultivars (Newkirk 2009) which should result in improved feed intake by hens. As noted in a review of the literature, GLS metabolism can cause problems including reduced iodine availability, impaired liver and kidney function, and morphological and physiological changes to the thyroid (Tripathi and Mishra 2007).

There is a need to re-evaluate the maximum recommended levels of CM as these were based on studies that are over 20 years old. The current recommended level for egg laying hens is a maximum of 10 % (Canola Council of Canada 2011). This maximum level could potentially be increased based on further improvements to newer cultivars of canola.

Juncea (*Brassica juncea*) is a type of mustard commonly grown in western Canada (Newkirk et al. 1997) mainly for the production of condiment mustard (Potts et al. 1999). Recently new varieties of juncea have been developed which meet the requirements to be of canola quality (Potts et al. 1999). The requirements are that there is less than 30 micromoles per gram (μmol/g) of GLS in the air-dry, oil-free meal, and less than 2 % erucic acid in the oil (Feeds Regulations 1983). Research by Newkirk et al. (1997) found that including 20 % canola-quality juncea meal (JM) in the diet of broiler chickens resulted in growth performance that was equal or superior to canola meal. Juncea meal has not been evaluated for use in white- or brown- shell egg laying hen diets.

Along with GLS, canola and juncea meals have other anti-nutritional factors which need to be dealt with, if these meals are be included in significant quantities in poultry diets. These anti-nutritional factors include fibre, which reduces the rate of

passage in the gastrointestinal (GI) tract and encapsulates nutrients (Choct 2002), and phytate, which binds phosphorus (P) and other nutrients making them unavailable for digestion and absorption (Newkirk 2009). Research found that including multicarbohydrase (Choct 2002) or phytase (Ravindran et al. 1999) can reduce the negative effects of fibre and phytate, respectively. Research needs to be conducted using canola and juncea meals, with the inclusion of dietary enzymes, to evaluate the appropriate maximum levels which can be used in both white- and brown- shell egg laying hen diets.

Chapter 2. Literature Review

2.1 Canola (*Brassica napus*)

Canola is a term which arose from "Canadian Oil" and is used to represent any variety of rapeseed that contains low levels of erucic acid (EA) and glucosinolates (Canola Council of Canada 2003). Originally, the term was used to distinguish these varieties developed in Canada from other common forms of rapeseed. Over time, however, the name became a global term and is now used to signify a variety of rapeseed that meets guidelines defined in the Canada Feeds Act. These guidelines state that to qualify as canola, the rapeseed must contain less than 2 % erucic acid in its oil and less than 30 μmol/g of glucosinolates in its air-dried oil-free meal (Feeds Regulations 1983). All varieties of rapeseed registered in Canada must be of canola quality unless stated that they are high EA or fatty acid (FA) varieties designed specifically for specialty markets (Canola Council of Canada 2003).

2.2 Juncea (*Brassica juncea*)

Plants other than rapeseed can be of canola quality. Juncea (*Brassica juncea*), a mustard seed in the same genus as canola (*Brassica*), also contains EA and GLS. Juncea can be recognized as canola quality as long as it meets the canola definition of less than 2 % erucic acid in the oil and less than 30 μmol/g of glucosinolates in the air-dried oil-free meal. Canola-quality juncea was first developed in 2002 by Agriculture and Agri-Food Canada (AAFC) Saskatoon, SK Research Centre and Saskatchewan Wheat Pool (SWP) (Canola Council of Canada 2003).

Juncea has some agronomic advantages when compared to *napus* canola including improved drought tolerance (Newkirk et al.1997), superior seedling vigour, more rapid ground covering ability, increased disease tolerance (especially blackleg caused by *Leptosphaeria maculans*) and enhanced pest tolerance (Gunasekera et al. 2006). Juncea contains the same anti-nutritional factors (GLS, EA, and tannins) as *napus* canola (Tangtaweewipat et al. 2004), although some of these have been overcome by breeding, at least in Australian varieties. Varieties have been bred that are erucic acid-free, have reduced glucosinolates, and have higher levels of the omega-6 essential fatty acids oleic and linoleic acids (Gunasekera et al. 2006).

2.3 Oilseed Processing

There are several ways that canola can be processed including pre-pressed solvent-extraction (PPSE), rolling, and cold-pressing. When PPSE (Fig. 2.1) is used the seeds are cleaned, pre-conditioned, flaked, cooked, and then mechanically pressed to remove some oil. The material leftover from the mechanical pressing (called the press cake) is then introduced to a solvent, usually hexane, which removes the remainder of the oil. Finally the solvent is removed from the resulting meal, which is then toasted, dried and cooled. PPSE is the most common commercial method when the canola oil is used as a source of edible oil or in the biofuel industry. The resulting meal (containing approximately 1-3 % oil) is often included in poultry diets (Newkirk 2009). Juncea, as an oilseed, can also be processed using PPSE (Bell et al. 1998).

While pre-pressed solvent extraction is an efficient method for removing oil from canola seeds, it can cause the quality of the canola meal to drop due to the temperatures and moisture conditions to which the meal must be exposed for the desolventization-toasting process. According to Newkirk et al. (2003a), Maillard browning reactions occur during this step, resulting in reduced amino acid content and digestibility resulting in reduced protein quality. However, since this method removes the most oil from the seeds, it is the method used by commercial crush operations, and therefore produces the majority of the CM available to producers for feeding. Canada's 14 crushing plants processed 8 million tonnes of seed which produced 3.9 million tonnes of PPSE canola meal during the 2011-2012 harvest (Canola Council of Canada 2011).

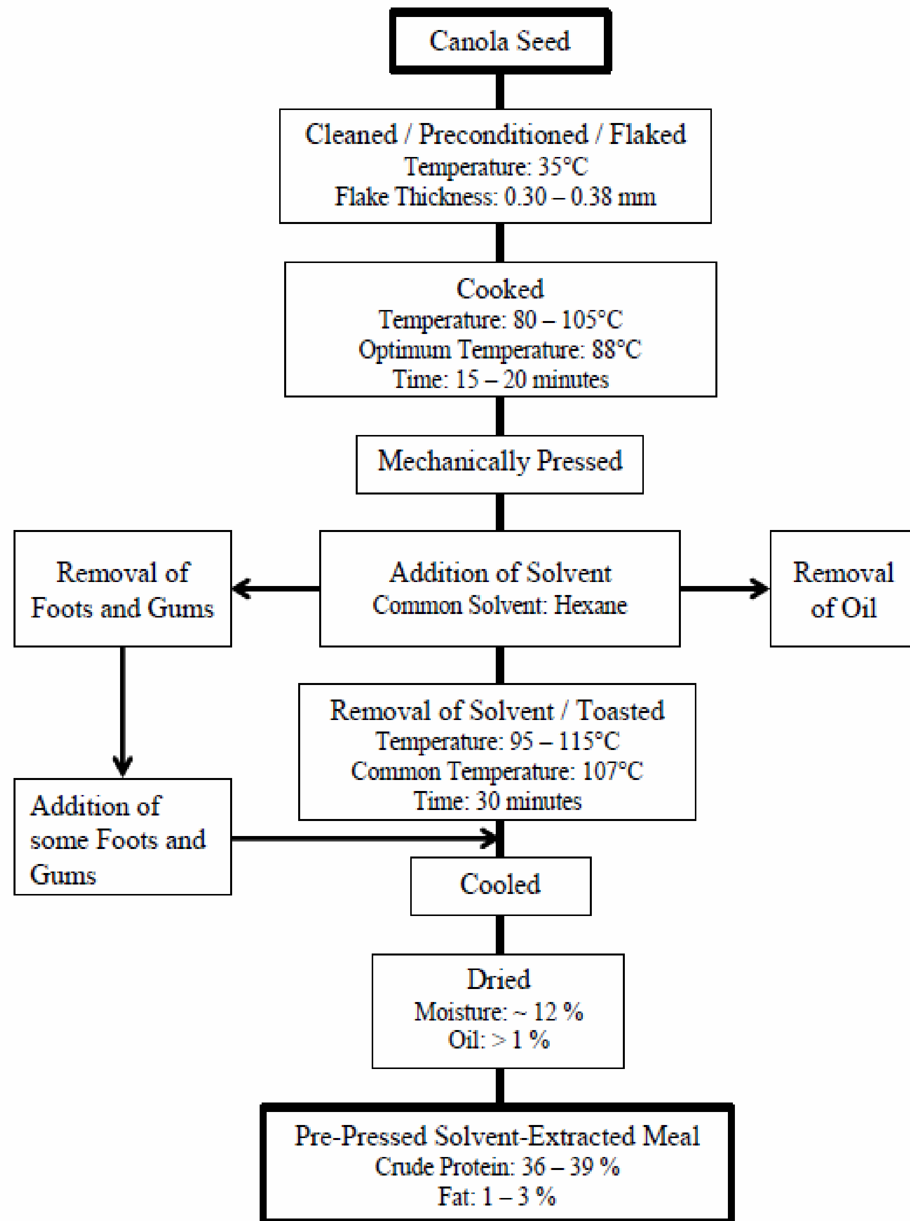

Fig. 2.1. Pre-press solvent-extraction (PPSE) of canola seed with data from Newkirk (2009).

2.4 Anti-Nutritional Factors of Canola and Juncea

Research with rapeseed meal (RSM) reported many negative effects on laying hen performance and egg quality including: reduced egg production (Leslie and Summers 1972, Olomu et al. 1975, Thomas et al. 1978 and Campbell 1979), reduced feed consumption (Summers et al. 1987), poorer feed conversion ratio (Marangos and Hill 1976), decreased egg weight (Marangos and Hill 1976 and Summers et al. 1987), decreased albumen height (in Haugh Units, Thomas et al. 1978), increased thyroid weight (Marangos et al. 1974, Olomu et al. 1975, Marangos and Hill 1976 and Thomas et al. 1978) and increased mortality rate (Marangos et al. 1974 and Olomu et al. 1975).

The limited research performed with varieties of mustard seed in laying hen diets resulted in similar negative effects on laying hen performance and egg quality, including: reduced egg production, reduced feed consumption, poorer feed conversion ratio (Cheva-Isarakul et al. 2001), decreased egg weight (Marangos et al. 1974 and Cheva-Isarakul et al. 2001) and increased mortality due to fatty liver hemorrhagic syndrome (Marangos et al. 1974).

Many of these negative effects can be related to the anti-nutritional factors present in CM and JM, which include glucosinolates, erucic acid, tannins, phytate, and fibre. There is not a single solution that can reduce or neutralize all of these components and some are commonly dealt with in more than one way. For example, breeding programs and heat treating of meal are both used to reduce the effect of GLS on animals consuming canola or juncea meals.

2.4.1 Glucosinolates

The term glucosinolate represents a wide variety of sulphur-containing secondary plant metabolites of which over 120 have been identified and named. All GLS share a sulphonated oxime moiety and a β-D-thioglucose group, but have a variable side chain (Fig. 2) derived from tryptophan, methionine, or phenylalanine (Tripathi and Mishra 2007).

Glucosinolates occur in plants as defence mechanisms against herbivorous pests (Stowe 1998). Environmental conditions can have a large impact on the GLS concentration found in plants. For example, water stress (drought) increased the GLS

concentration in *Brassica napus* (Mailer and Cornish 1987) and other cruciferous plants (Ciska et al. 2000) when compared to the same species of plant, grown with adequate water. This can result in varying GLS concentrations from the same species of *Brassica* grown in the same place at different times (Ciska et al. 2000).

The major GLS present in CM are gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin, and 4-hydroxyglucobrassicin (see Fig. 2.2 for structures; Khajali and Slominski 2012). Concentrations of specific GLS differ according to the particular plant tissue being tested, with seeds having a higher amount of progoitrin than other tissues (Mithen 1992). The GLS in juncea meal tend to be mostly alkenyl GLS (Krumbein et al. 2005), specifically sinigrin (R = allyl-) and 4-hydroxyglucobrassicin (R = 4-hydroxy-3-indolylmethyl-) (Sang et al. 1984). Newkirk et al. (1997) found that *B. juncea* contained more aliphatic GLS than *B. napus* (24.2 versus 11.5 µmol/g), and that this difference was due to the higher content of gluconapin in *B. juncea* (21.2 versus 3.4 µmol/g).

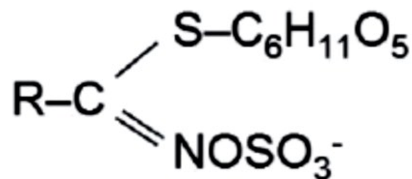

Fig. 2.2. General structure of glucosinolates (GLS) from Khajali and Slominski (2012).

The R- group changes for each type of GLS. The name and structure of the most abundant GLS in canola are as follows: gluconapin (R = 3-butenyl-), glucobrassicanapin (R = 4-pentenyl-), progoitrin (R = 2-hydroxy-3-butenly-), gluconapoleiferin (R = 2-hydroxy-4-pentenyl-), glucobrassicin (R = 3-indolylmethyl-), and 4-hydroxyglucobrassicin (R = 4-hydroxy-3-indolylmethyl-).

Bjerg et al. (1989) reported that while GLS themselves can have negative effects on animals, the breakdown products that result from the hydrolysis of GLS amplify these negative effects. Therefore, hydrolysis should be prevented if possible. Hydrolysis is initiated when the seed is cracked (ruptured) and moisture enters (Morra and Kirkegaard 2002). The enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), is found in the seed (Höglund et al. 1991) but is stored separately from the GLS (Bones et al. 1991). Rupture of the seed causes myrosinase to come into contact with GLS, producing an

unstable aglucone. This aglucone continues to react, but the end product formed depends on the conditions of the reaction (pH, the presence of ions or ascorbic acid) and the original side chain of the GLS (Bones and Rossiter 1996, Fig. 2.3).

pH 7 → $RNCS + SO_4^{2-} + glc$
isothiocyanates

pH 4 or Fe^{2+} → $RCN + S + SO_4^{2-} + glc$
nitriles

S—Glc / R— / $NOSO_3^-$
glucosinolate

H_20 + myrosinase →

S^- / R— / $NOSO_3^-$
unstable aglucone

ESP and Fe^{2+}
R = alkenyl
→ CN + SO_4^{2-} + glc
cyanoepithioalkanes

R = allyl-
R = benzyl-
R = 4-(methylthio)butyl-
→ $RSCN + SO_4^{2-} + glc$
thiocyanates

Fig. 2.3. Hydrolysis of glucosinolates modified from Bones and Rossiter (1996).

All glucosinolates react to form unstable aglucones in the presence of myrosinase and moisture. At a neutral pH, isothiocyanates are formed. In the presence of iron ions or at a low pH (4) nitriles are produced. Cyanoepithioalkanes occur in the presence of epithiospecific proteins (ESP) and iron ions, and when the side chain is alkenyl. Thiocyanates are only produced when the side chain is allyl-, benzyl- or 4-(methylthio)butyl-.

The end products of GLS hydrolysis can include isothiocyanates (responsible for bitterness), and nitriles, thiocyanates, thiourea and oxazolidithione, which disrupt thyroid function by reducing the availability of iodine (Tripathi and Mishra 2007). These products may be responsible for some of the negative effects that have been associated with canola use, as studies done with high-GLS RSM have found reduced egg production (Smith and Campbell 1976 and Thomas et al. 1978), reduced egg quality (Haugh units) and marked enlargement of the thyroid (Thomas et al. 1978) when hens consumed these meals.

Two solutions for dealing with GLS in canola and juncea meals are to reduce GLS concentration through breeding programs and to treat the meals with heat to deactivate the myrosinase enzyme. Breeding programs have currently produced both canola (Stefansson and Kondra 1975) and juncea meals (Love et al. 1990) with GLS levels well below the 30 µmol/g required to be considered canola quality, however there have still been reports of fatty liver hemorrhagic syndrome (FLHS, Campbell 1979) and thyroid hypertrophy (McKinnon and Bowland 1979) when laying hens and young pigs, respectively, consume these low GLS meals.

There are several possible ways to heat-treat canola seeds. Eapen et al. (1968) heat treated *Brassica napus* seeds by dry heat (jacketed pan or hot-air oven), steam-blanching, microwave heating, or wet heat (soaking in boiling water or soaking in boiling water then wet-grinding in a vertical plate grinder). This study found that dry heat did not destroy the myrosinase enzyme completely, meaning that GLS hydrolysis could continue, resulting in the formation of breakdown products. While steam-blanching, microwave heating, and soaking all inactivated the myrosinase enzyme, there was some degradation in the quality of the meal (for microwave heating) and oil released when these heat treatment methods were used (Eapen et al. 1968).

Newkirk et al. (2003b) found that toasting the meal of brown-seeded canola actually reduced the GLS content from 10.55 to 6.16 µmol / g of the meal, but it also reduced the amino acid content and protein digestibility of the meal. Newkirk et al. (2003b) suggested that the detrimental effects of toasting the meal outweighed any benefit of reduced GLS content, since the GLS level of the meal was low before toasting occurred.

When commercial dry-heat treatments were compared with wet-heat treatments, it was found that dry-heat was as effective as wet-heat at deactivating the myrosinase, but that wet-heat produced a better quality meal (higher in protein) and oil that was lighter colour and had less free-fatty acids (Eapen et al. 1968).

2.4.2 Erucic Acid

Erucic acid (C22:1 n-9) is a long chain mono-unsaturated FA found in the oil portion of rapeseed and canola. EA is a concern because some studies found an increase

in the EA content of triglycerides, and a build-up of these triglycerides in the heart muscle (Beare-Rogers and Nera 1972), and an increase in heart muscle degenerative lesions of rats (Beare-Rogers and Nera 1972 and McCutcheon et al. 1976). Findings such as these caused concern about what effects EA could have on human heart health as well, leading to studies such as the one conducted by Green and Innis (2000) which found that including canola oil in infant formula caused EA accumulation in triglycerides, but did not increase the level of triglycerides accumulating in the heart. These results indicate that low levels of EA do not cause damage to human heart tissue.

A study by Vogtmann et al. (1974) with laying hens fed high EA (26.2 %) rapeseed oil (HEA) and low EA (4.1 %) span rapeseed oil (LEA) at either 5 or 15 % of the diet, and compared it to soybean oil fed at the same levels. The study found the HEA, fed at 15 % reduced feed consumption and egg production of the hens. Total egg weight and yolk weight were also reduced when feeding HEA at 15 %, but the percentage of lipid in the yolk, and the percentage of FAs in the lipid were unaffected by dietary treatment (Vogtmann et al. 1974). In the same study the feed and egg yolks were analysed for total FA composition. It was found that the 5 % HEA diet gave the hens 15.7 % EA (as a percentage of total FA present in the diet), when the amount of EA provided by all feed ingredient (not just the oil) was accounted for. Similarly, the 15 % HEA diet gave the hens 22.1 % EA as a percentage of the total FA present in the diet. However, less than 0.1 % (trace) or 0.2 % EA was present in yolks from hens consuming the respective diets. Similar results were found for the 5 % (providing 2.5 % EA as a percentage of the total FA present in the diet) or 15 % (providing 3.8 % EA) LEA diets. No EA was present in the yolks from these hens (Vogtmann et al. 1974).

These results indicate that while high levels of EA are detrimental to egg production, the eggs produced by hens consuming EA will not contain enough EA to be a danger to human health. Therefore, a reduction in EA content of CM or JM would be beneficial for increased egg production and egg quality.

This reduction in EA was achieved through plant breeding programs. A review of the literature found that the EA content of *Brassica napus* was reduced from 56.3 % (rapeseed) to 0.3 % (canola). The EA content of *Brassica juncea* was also reduced from 46.6 % to 0.3 % (McVetty and Scarth 2002) allowing it to meet the canola quality

standards. Leeson et al. (1987b) found that completely replacing the SBM with CM in a laying hen diet resulted in only trace amounts (less than 0.1 %) of EA in the egg yolks (confirming the results of Vogtmann et al. 1974), and had no detrimental effects on feed consumption, egg production or egg quality.

2.4.3 Tannins

In a review of the literature Khajali and Slominski (2012) describe tannins as complex polyphenolic compounds which can be subdivided into hydrolysable and condensed (insoluble) fractions. As a group, tannins have been found to form complexes with enzymes (Goldstein and Swain 1965) and proteins (Calderon et al. 1968), making them unavailable to the hen. Oh et al. (1980) found that the number of methylene groups in the amino acid side chain determines the strength of the protein-tannin complex. Tannic acid (water-soluble tannins) has been shown to reduce egg production and feed consumption when included in the diet at 2 or 4 % (Blakeslee and Wilson 1979) and proanthocyanidines (the insoluble fraction) have been shown to decrease egg production and egg weight (Guillaume and Bellec 1977). Tannins are found in the seeds of *Brassica napus* (Bate-Smith and Ribéreau-Gayon 1959), specifically, in the seed hulls (Naczk et al. 1994). Because the majority of the tannins present in canola hulls are insoluble, (Khajali and Slominski 2012) and tannins account for only 0.1 % of the hull overall (Leung et al. 1979), the anti-nutritive effect of canola tannins would be almost negligible.

2.4.4 Phytate (Phytic Acid)

Phosphorus in animal tissues exists only in the form of phosphate, which can be supplied by grain (for omnivores and herbivores) or the soft tissues and bones of other animals (for carnivores). Phosphates in plants come from P in the soil, and are at least partially bound as phytate, reducing the availability of phosphates to herbivorous animals (Sjaastad et al. 2003).

Phytate is a mixed salt (consisting of potassium, magnesium and calcium) of phytic acid. It is considered the principal storage form of P in oilseeds (Pallauf and Rimbach 1997) but is an anti-nutritional factor for animals because it forms complexes with minerals (calcium, iron, zinc, manganese and magnesium) and proteins. As well, the

chemical structure of phytate allows it to chelate (combine with a metal ion to form a ring) with cations, making them unavailable to the animal. Phytate can also change sodium partitioning within the body. This can affect the sodium-dependent transport of certain nutrients (glucose and peptides) from the gut (Khajali and Slominski 2012).

The total amount of P in CM is around 1.13 % (on a 100 % dry matter basis) with approximately 37.2 % of that being non-phytate phosphorus (NPP), which is available to the animal. The other 62.8 % is phytate P, which is not available to the animal. The amount of phytate P in CM is higher than that of SBM which contains 0.73 % P (on a 100 % dry matter (DM) basis) with 57.5 % of that being phytate P (modified from a review by Khajali and Slominski 2012). Few studies have reported the nutrient composition of JM, but a study by Bell et al. (1984) found that brown-seeded juncea meal had a total amount of P similar to that of the CM used in the study (1.25 % P and 1.33 % respectively, on a 100 % DM basis). The amount of phyate P was not reported.

A solution to the problems caused by phytate is to include a phytase enzyme in the diet. A commercially available form of phytase is Bio-Phytase 5000G (Canadian Bio-Systems Inc. Calgary, Alberta). For poultry, 75-250 grams of Bio-Phytase can be included per tonne of complete feed, providing a minimum of 5000 phytase units (FYT) per gram. One FYT equals the amount of enzyme (from *Aspergillus oryzae*) required to release 1µmol of inorganic phosphate per minute at 37ºC with a pH of 5.5. The range in values provided account for the fact that some pelleting and conditioning processes may reduce the detectable enzyme activity in feed.

While phytase is known to enhance the availability of phosphorus, research by Ravindran et al. (1999) shows that supplementation with microbial phytase (1200 FYT/Kg) may also enhance amino acid digestibility. This is important because heat treating is a common way to deal with the anti-nutritional factors of GLS due to hydrolysis by the enzyme myrosinase. This can result in reduced amino acid digestibility as well because of Maillard reactions caused during heating (Newkirk et al. 2003a).

2.4.5 Fibre

Carbohydrates are found in all plant material, with the majority of the carbohydrate as starch which is highly digestible for poultry. Carbohydrates can occur in

other forms such as polysaccharides and oligosaccharides (National Research Council, NRC 1994) which are collectively called non-starch polysaccharides (NSP) and make up what we know as fibre. NSP can be divided into two categories: soluble (partially soluble) and insoluble.

Soluble NSP can reduce the metabolisable energy (ME) of a diet due to increased microbial fermentation. Fermentation produces energy in the form of heat and volatile fatty acids, some of which can still be absorbed by the animal, while the rest are lost to the surrounding environment through excreta. Energy which was a product of microbial fermentation (conversion of starch to volatile fatty acids) cannot be used as efficiently as energy which was directly absorbed from the intestine. This loss of energy means that diets high in soluble NSP tend to have ME values lower than expected based on the amount of energy available in the feed (Choct 2002). Lee et al. (1991) found that diets containing flaxseed oil had a higher ME than diets containing whole flax seed, when the oil to meal ratio was similar between the two diets. From an average of several data sets in the literature, Khajali and Slominski (2012) found that CM had a lower ME than SBM even though CM has a higher oil content, which should off-set the fact that SBM is higher in oligosaccharides that can be converted to energy in the form of short-chain FAs by GI microbes. This demonstrates how the addition of fibre and NSP can reduce the ME by creating a barrier which poultry cannot efficiently breach to make all the energy available.

Meals made from canola or juncea contain hulls which account for a large part of the seed (Newkirk 2009). The hulls are predominantly fibre (Naczk et al. 1994), specifically insoluble NSP (Bell and Shires 1982), and account for 30 % of meal weight (Bell 1993). NSPs can have an effect on the viscosity of the digesta, and the physiology of the digestive tract. Fibre causes thickening of the mucosa of the gastrointestinal tract which may result in diminished ability of the intestinal wall to absorb nutrients (Choct 2002).

One advantage of JM compared to CM is that JM has been found to have less dietary (total) fibre. However, it has also been reported that while JM has less total fibre than CM, it has more NSP in the fibre fraction (Simbaya et al. 1995 and Slominski et al.

1999). This may explain why JM performed in a similar manner to CM in terms of body weight gain and gain to feed ratio in a study with broiler chickens (Newkirk et al. 1997).

There are several ways to reduce the anti-nutritional effects of fibre in diets for monogastric animals. Grinding CM can help improve the energy and protein digestibility (Bell et al. 1985), but the addition of carbohydrase enzymes may result in the breakdown of NSP which could further increase energy availability (Meng et al. 2005). Enzymes such as xylanase can help improve the reduced absorption of nutrients observed when fibre is fed by reducing the large NSP molecules into smaller polymers, and reducing the digesta viscosity as well (Choct 2002).

A commercially available form of an enzyme cocktail with mixed carbohydrases is Superzyme-OM™ (Canadian Bio-Systems Inc. Calgary, Alberta). For poultry it is recommended that 500 grams of Superzyme-OM™ be included per tonne of complete feed, providing a minimum of 2800 cellulase units (CMC), 400 mannanase units (MAN), 50 galactanase units (GAL), 1000 xylanase units (XYL), 600 glucanase units (GLU), 2500 amylase units (FAA), and 200 protease units (HUT) per gram. Superzyme-OM™ contains a minimum of 12% crude protein, a maximum of 5 % crude fibre and 11 % moisture. The enzymes are from several sources including dried fermentation extract from *Aspergillus oryzae*, *Aspergillus niger*, and *Trichoderma reesei*, and dried fermentation solubles extract from *Saccharomyces cerevisiae*.

2.5 Egg Quality Factors

The egg of the laying hen consists of three major sections: the shell and its membranes, the yolk, and the albumen (egg white). The formation of these sections involves a number of highly complex steps that convert nutrients from the feed into nutrients to be packaged into the egg. The nutrients required for the egg can total more than 3 % of the hen's body weight, and include, on average, 7.7 g of protein, 7.0 g of lipids, 2.0 g of calcium and 40 g of water (Burley and Vadehra 1989).

The quality of the egg depends largely on the conversion of the nutrients in the feed to the egg constituents, and the way in which the hen puts the constituents together. There are some other factors that are not really 'quality' issues, so much as consumer preferences. These factors can include characteristics like ratio of yolk to albumen, and

the colour of the egg yolk or shell. While these factors do not affect the quality of an egg, they can affect whether or not the consumer will purchase those eggs. For this reason these factors are monitored along with true measures of quality.

2.5.1 Egg Formation in the Laying Hen

Egg formation in the laying hen begins in the left ovary, which is attached to the dorsal abdominal wall, between the left lung and kidney. The follicles found on the ovary develop in sequence (with the first follicle approximately 24 hours ahead of the second one) in sexually mature hens, so that it is possible to identify between 5 and 6 follicles, at different stages of development, at any given time (Sjaastad et al. 2003). Follicles, which consist of the ovum and the membranes that encase it (Solomon 1997), begin as an oocyte surrounded by an inner layer of granulosa cells and an outer layer of theca cells (Sjaastad et al. 2003). The active ovary produces hormones, including estrogen, which signal the liver to form low density lipoproteins (LDL) from a combination of fats and proteins (Bell and Weaver 2002). These lipoproteins are transported through the blood, to the ovary, where they are deposited in the follicle, along with water, minerals, and vitamins to form the yolk (Sjaastad et al. 2003). The LDLs account for about 60 % of the dry weight of the yolk (Bell and Weaver 2002). Over the course of a hen's active laying life, between six and seven hundred mature yolks will be produced (Burley and Vadehra 1989).

The mature ovum hangs from the ovary on a stalk containing arteries. The follicle is highly vascular, except for the stigma (a narrow area around the yolk that is almost void of blood vessels). Progesterone from the active ovary triggers the hypothalamus, which stimulates luteinizing hormone to be released from the anterior pituitary (Bell and Weaver 2002). Luteinizing hormone initiates ovulation of the ovum (Sjaastad et al. 2003) by causing the follicle to rupture at the stigma (Bell and Weaver 2002). This rupture is usually free from hemorrhage, although occasionally a tear will occur, causing a blood spot to be deposited onto the yolk (Solomon 1997). Once the yolk is released from the ovary, the vitelline membrane surrounds it (Bell and Weaver 2002).

The yolk released from the ruptured follicle is caught by the oviduct (Fig. 2.4), which is made up of five distinct parts (the infundibulum, the magnum, the isthmus, the

uterus, and the vagina), each with a specific function for egg formation. The infundibulum is the first section of the oviduct, and is about 9 cm long (Bell and Weaver 2002). The broad funnel-shaped ampula, located at the anterior end (Burley and Vadehra 1989), is made up of smooth muscle so that it can change position or shape allowing it to surround the oocyte once it has been released from the follicle. This ensures the oocyte will be caught (Sjaastad et al. 2003) and deposited into the chalaziferous (tubular) region of the infundibulum where the outer layer of the vitelline membrane (Fig. 2.5) and the chalazal layer of the albumen are added to the yolk (Burley and Vadehra 1989) . The oocyte remains in the infundibulum for approximately 15 minutes before it enters the magnum (Sjaastad et al. 2003).

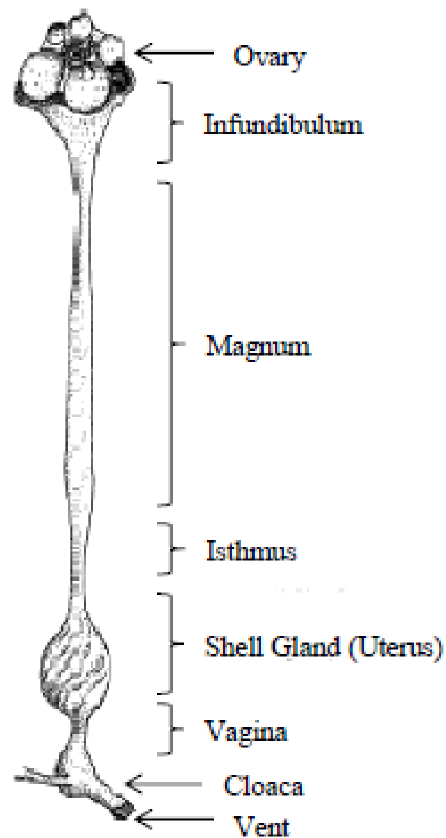

Fig. 2.4. Laying hen reproductive tract modified from Roberts (2004).

The magnum (the second part of the oviduct, 33 cm long) is where the rest of the albumen is added to the developing egg (Bell and Weaver 2002). The albumen is

comprised of 40 different types of protein (Solomon 1997) which are either produced in the glands of the magnum (stimulated by estrogens and progesterone) or are constructed in the liver, and transported through the bloodstream to the magnum (Sjaastad et al. 2003). The proteins are deposited onto the yolk in layers (Sjaastad et al. 2003) where they function as an antibacterial buffer, act as an outline for the deposition of shell membranes, and protect the yolk from physical damage (Solomon 1997).

There is only one type of albumen produced in the magnum (the dense white), but as the forming egg passes along the oviduct it is rotated and water is added. These additions result in four distinct types of albumen in the fully formed egg, each with a slightly different consistency: dense white, liquid inner white, outer thin white and the chalazae (Fig. 2.5). The dense white contains mucin that helps to hold it together. Over time, with the breakdown of mucin and the addition of water, the thin outer white is formed. When the egg is laid, there will be 3 times more thin outer albumen than was originally deposited in the magnum, but the thin white will still account for less than half of the total albumen (Bell and Weaver 2002).

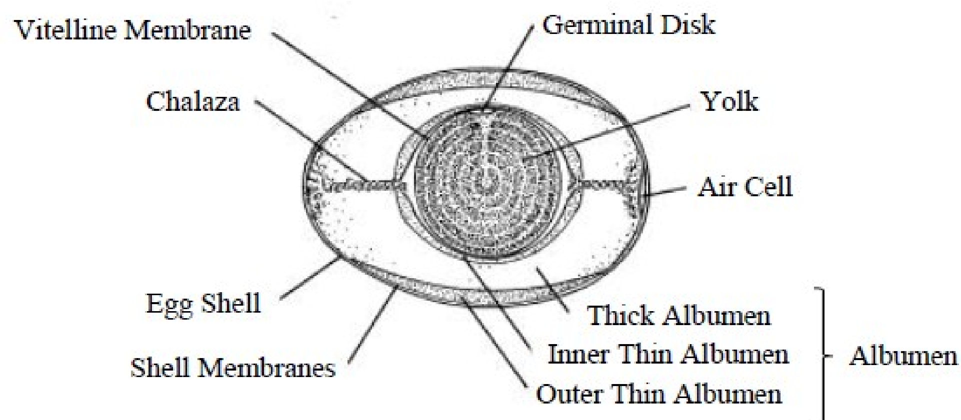

Fig. 2.5. Diagram of fully-formed egg from Roberts (2004).

The chalazae are two chords that hold the yolk in the centre of the egg (Fig. 2.5). The albumen is formed when the yolk enters the magnum, but is not twisted to form the chords until the egg rotates in the lower part of the oviduct (Bell and Weaver 2002). The

process of adding the albumen to the egg yolk takes about three hours (Sjaastad et al. 2003).

Once the albumen has been added to the ovum, it enters the isthmus, which is the 10 cm long section of the oviduct where the shell membranes are added. Keratin filaments are secreted from glandular cells located in the isthmus (Sjaastad et al. 2003). These filaments surround the albumen, forming two strong membranes (Sjaastad et al. 2003) which act as a barrier to bacteria and give the egg it`s final shape (Bell and Weaver 2002). The inner membrane is deposited onto the albumen first, and is thinner than the second, outer membrane. When the membranes are added to the forming egg, the contents of the egg do not fill them so that the egg now resembles a sack that is only partly filled. This process of adding the membranes takes approximately 1.5 hours (Bell and Weaver 2002).

When the egg leaves the isthmus, it enters the uterus which is a 10 to 12 cm long section of the oviduct, also known as the shell gland. Here, water and salts (used to plump the membranes and liquefy some of the albumen to form the outer thin white) enter the shell membranes through osmosis, the shell is formed, any pigment is added, and the cuticle is laid down (Bell and Weaver 2002).

Egg shell formation is the longest part of the process, and takes approximately 15 of the 18 to 20 hours that the egg remains in the uterus. The shell (Fig. 2.5), made from calcium carbonate and small amounts of sodium, potassium, and magnesium, is added to the outer shell membrane (Sjaastad et al. 2003) in two layers. The spongy inner layer is composed of calcite crystals, attached at initiation sites that form just before the egg enters the shell gland. The chalky, outer layer is added on top of the first layer, and contains columns of calcite crystals that are about twice as long as the first layer. The longer the columns are, the stronger the shell is (Bell and Weaver 2002).

Prior to egg-laying, hens form trabecular bone tissue in the marrow cavity of the long bones. Calcium (Ca) is mobilized from the trabecular bone and (along with dietary Ca) is carried through the blood to the oviduct. Approximately 2 g of Ca are transported across the epithelial cells, into the lumen of the oviduct during the 15 hours it takes to form the shell. Carbon dioxide and water in the shell gland are catalyzed into carbonic acid by carbonic anhydrase. This causes the calcium carbonate crystals to form

spontaneously on the outer shell membrane. If the hen is a brown shelled layer, the pigment (called porphyrin, formed from heme) is added during the process of shell formation (Sjaastad et al. 2003).

Once the eggshell is formed, the cuticle is deposited on the outside of the shell. The cuticle is a thin membrane made up of proteins that are secreted by epithelial cells at the distal end of the shell gland (Sjaastad et al. 2003). The cuticle contains water, which allows it to act as a lubricant during oviposition (laying). When the cuticle dries on the egg that has been laid, it seals the pores in the shell, helping to prevent the entry of bacteria (Bell and Weaver 2002).

The last section of the oviduct is the vagina, which is about 12 cm long. The vagina does not have a function in egg formation, but allows the fully formed egg (Fig. 2.5) to be expelled. Eggs are rotated horizontally in the vagina to allow them to be laid large end first, unless the hen is frightened just prior to laying. This is done to give the uterine muscles more surface area to contract against, making it easier to push the egg out (Bell and Weaver 2002).

2.5.2 Complications During Egg Formation

There are several complications that can occur during egg formation and laying that can cause a decrease in egg production or egg quality. A malfunction of the infundibulum can cause between 4 and 10 % of yolks ovulated to not be drawn into the opening of the oviduct (Bell and Weaver 2002). Infections or adhesions of the infundibulum can reduce the size of the opening, making it harder to catch the yolks that were ovulated. These yolks will remain in the body cavity of the laying hen, and will eventually be resorbed (Sjaastad et al. 2003). It is also possible for the infundibulum to lose its ability to catch a high number of the ovulated yolks. These yolks would build up in the body cavity faster than they can be resorbed, causing the hen to be dubbed an ‘internal layer’ (Bell and Weaver 2002). Both of these malfunctions would result in an egg not being laid on that day, decreasing production numbers, but not affecting the quality of successive eggs laid.

There are several complications during egg formation that can affect the eggshell quality. If an egg did not spend enough time in the shell gland, it may be laid with a soft

shell. If an egg is cracked during formation but is still in the shell gland, the crack (or body check) will be covered with a new layer of shell over the cracked area. This usually leaves a ridged area on the shell. Eggs can also be laid with thin shells (Bell and Weaver 2002). This can be caused by nutritional (inadequate Ca or Vitamin D (Vit D) in the diet causing impairment of calcium carbonate formation) or environmental (temperature) factors, and also by disease or genetics. Inadequate dietary Ca leads to reduced Ca storage in the medullary bone, reducing the amount of Ca available for calcium carbonate (shell) formation (Sjaastad et al. 2003).

Inadequate dietary Vit D can cause problems with Ca transport into the blood from the GI tract, and from the blood to the epithelial cells of the shell gland. This is because calcitriol, the active form of Vit D, is responsible for promoting the formation of Ca binding proteins that aid in the transport of Ca (Sjaastad et al. 2003).

In both cases, inadequate Ca causes carbonic anhydrase production in the epithelial cells of the shell gland to be repressed, resulting in insufficient secretion of bicarbonate into the lumen of the uterus. This lack of bicarbonate impairs calcium carbonate formation and leads to weak eggshells (Sjaastad et al. 2003).

Environmental factors such as high temperature can cause thin eggshells during formation for several reasons. One reason is due to physiological changes that occur within the hen because of increased respiration. Another reason is due to a reduction in feed intake, causing a reduction of Ca, and therefore inhibiting calcium carbonate formation (Bell and Weaver 2002).

Disease can produce weak shells for reasons similar to those for high temperature, especially a respiratory disease such as infectious bronchitis or Newcastle disease (Bell and Weaver 2002).

2.5.3 Egg Quality Measurements

There are several measurements that have been developed as indicators of egg quality. They are based on factors that are important to the egg industry (Stadelman 1995) and the consumer (Wells 1968) such as egg specific gravity (SG), egg weight, shell breaking strength, shell weight, albumen height and yolk weight. Nutrition can affect these factors, sometimes significantly altering them.

Egg weight is used by the industry in conjunction with egg shape (eggs are usually ovoid in shape, but some abnormalities in the oviduct can cause eggs that are round, long, wrinkled, ridged, flat-sided or pointed (Bell and Weaver 2002)), shell appearance (dirt, cracks, colour), yolk appearance, runniness of albumen, and the presence of blood or meat spots (all determined by some form of candling) to assign a grade to the eggs (Stadelman 1995). Individual weights can be used to evaluate specific eggs, which would help to determine the effect that a diet change may have on egg formation. Normal egg weights for the Canadian industry include jumbo (greater than 70 g), extra-large (64-69.9 g), large (56-63.9 g), medium (49-55.9 g), small (42-48.9 g) and peewee (less than 42 g) (Alberta Egg Producers 2010).

Rowghani et al. (2007) found that including 3 and 5 % canola oil in the diet of laying hens had no effect on egg weight. These results were similar to those from Najib and Al-Khateeb (2004) where whole canola seed was included in the diet up to 10 % with no effect on egg weight, and to Leeson et al. (2007) who fed flaxseed at 10 % with no deleterious effects. Jia et al. (2008) found that the inclusion of a multi-carbohydrase enzyme (Superzyme-OM™) in a 15 % canola seed diet had no significant effect on egg weight.

Shell strength is one of the most important quality elements to the table egg industry because the whole industry relies on the ability of the egg to reach the consumer intact. Broken eggs are a source of great economic loss to the industry (Stadelman 1995). There are several factors that can be used as a measure of shell strength, including both direct (quasi-static compression, impact tests or puncture tests (Bain 1997)) and indirect (specific gravity, shell weight) methods (Roberts 2004).

Direct measures of egg shell strength require special recording equipment, which can measure and record the force applied to the egg when failure (shell breakage) occurs. These direct measures were devised to mimic the types of damage that eggs may encounter in a production setting, but are destructive, and therefore, can only be used to test one section of the shell. For comparison reasons, it is important that the same area of each egg is tested (Bain 1997).

Impact tests measure force in terms of the height from which an object (usually a steel ball of a known weight) must be dropped to fracture the shell. This can be done

either by dropping the ball from various heights until the shell breaks, or by dropping the ball repeatedly from the same height, recording the number of drops it takes to fracture the eggshell (Bain 1997).

A puncture test is the only type of destructive test that allows more than one measurement to be made on the same egg. For this test a punch (with a constant, known punch speed) is applied to the shell, and the force required to breach the shell is recorded (Bain 1997).

Quasi-static compression (the method used in this experiment) applies force to the egg by compressing it between two plates. A steadily increasing amount of force is applied until failure occurs (Bain 1997). The minimum force necessary to crack the shell is recorded (Roberts 2004). Normal eggs should be able to withstand 3 to 4 kg of force before breaking (Bell and Weaver 2002). This corresponds to breeder references which indicate that eggs from Lohmann LSL-Lite laying hens should have an egg shell breaking strength of 40 Newton, while eggs from Lohmann Brown laying hens should be over 35 Newton (Lohmann Tierzucht 2010), or approximately 4.08 kg force and over 3.57 kg force, respectively.

Specific gravity of freshly laid eggs has been found to closely correlate with shell thickness (Stadelman 1995). There are two ways to measure SG: using Archimedes Principle, or by flotation in saline solutions. Archimedes principle requires the egg to be weighed in air, then submerged and weighed a second time in water (Bain 1997). For the flotation method a hydrometer is used to calibrate saline solutions in increasing graded levels. The eggs are then placed in the solutions starting at the lowest concentration moving to the higher concentrations until they float. Eggs are assigned the SG of the first solution they float in. It is important to test freshly laid eggs as storage allows the development of an air cell, which can change the concentration of saline needed to float the egg (Stadelman 1995). Normal eggs will have a specific gravity that ranges between 1.070 (for a thin shelled egg) and 1.090 (for a thick shelled egg), with an average of 1.080 (Bell and Weaver 2002).

Egg shell weight is determined by breaking open the egg removing the yolk and albumen, and washing the shell out carefully so that no small pieces are lost. The shell is air dried and weighed. The percentage of shell can then be calculated by comparing the

shell weight to the weight of the whole egg. Typical eggs consist of 9 to 12 percent shell (Roberts 2004). Eggs with higher percent shell are said to be stronger than those with lower percent shell, at the same total egg weight.

Najib and Al-Khateeb (2004) found that whole canola seed could be fed up to 30 % in the diet with no effect on egg SG. In 2007, Leeson et al. found that flaxseed could be included in the diet at 10 % with no negative effects on shell deformation.

Albumen height is an important internal quality measurement for shell eggs because when consumers break an egg open (especially an egg to be used for frying) they want to see a good proportion of thick albumen, that will hold its shape well (Wells 1968). Albumen height is measured by calculating the thickness of the albumen (in millimeters) approximately one centimeter from the yolk (Roberts 2004). Albumen quality can be expressed in terms of height (mm) or in Haugh Units (HU) which relates the height of the albumen in millimeters to the weight of the whole egg in grams. Albumen height can be converted to HU using the following formula:

$$HU = 100LOG\ (H - 1.7W^{0.37} + 7.6)$$

Where H is the observed height of the albumen in millimeters and W is the weight of the egg in grams (Eisen et al. 1962 and Lokaewmanee et al. 2011). The table egg industry in Canada uses HU as part of the grading process. Eggs can be graded Canada Grade A if they receive a HU score of at least 67 (Egg Regulations 2009). Najib and Al-Khateeb (2004) found that when whole canola was fed at 30 % of the diet for laying hens, there was an increase in albumen height. Thomas et al. (1978) found that feeding low-glucosinolate RSM at 15 % of the diet did not affect HU, but feeding 10 and 15 % high-glucosinolate RSM caused a significant decrease in HU.

Yolk weight is another measure of internal egg quality but it is not a common one. It is important to this study, however, because the addition of fat and/or protein in the diet can increase the yolk size (Bell and Weaver 2002). According to the review paper by Roberts (2004), typical eggs have a yolk that accounts for 30 to 33 percent of the total egg weight. In 2007, Rowghani et al. found that feeding canola oil at 5 % to laying hens caused an increase in yolk weight.

2.6 Bone Quality Factors

There are three types of bone found in female poultry: cortical, trabecular (cancellous), and medullary. Cortical bone is a highly organized, tightly packed structural bone while trabecular bone, found at bone ends, has a three-dimensional honeycomb structure. Medullary bone is found in the marrow cavities of bones and develops in response to blood hormone levels of sexually mature hens (Kim et al. 2012).

2.6.1 Bone Formation

Bone formation (Fig. 2.6) begins while the chick is in the egg and is not subject to mechanical stress. Bones begin as cartilage (Fig. 2.6a), which forms in the shape of the bone it will become through ossification. The rate of ossification increases during incubation, but the skeleton of the newly hatched chick still has large amounts of cartilage that need to be converted to bone (Fig. 2.6b). This occurs during the growing period of the animal, and once ossified, bones will continue to grow after the animal reaches sexual maturity (Sjaastad et al. 2003).

There are two types of bone growth in long bones; longitudinal growth through endochondral ossification and bone widening through intramembranous ossification. Longitudinal growth (Fig. 2.6c) begins in the middle of the long bone at the primary ossification centre (Sjaastad et al. 2003). Here, resting chondrocytes in the germinal layer replicate and form columns densely packed into an extracellular matrix (ECM, Whitehead 2004) which contains a large amount of type II collagen (Velleman 2000) secreted by the chondrocytes (Whitehead 2004). The cells in this area, called the zone of proliferation, become less tightly packed within the columns as more ECM is secreted. The chondrocytes begin to differentiate and enter a hypertrophic state, where cells become enlarged, more rounded (Whitehead 2004), and begin secreting a new ECM component, type X collagen (Velleman 2000). The area where this occurs is called the hypertrophic zone. There exists pre-hypertrophic chondrocytes in the area between the zone of proliferation and the hypertrophic zones. This area is non-vascular, as proliferative chondrocytes receive nutrients from the epiphyseal capillaries and hypertrophic chondrocytes from the metaphyseal blood vessels. Chondrocytes secrete

other ECM components including proteoglycans and growth factors. These components of the ECM regulate further chondrocyte development (Whitehead 2004).

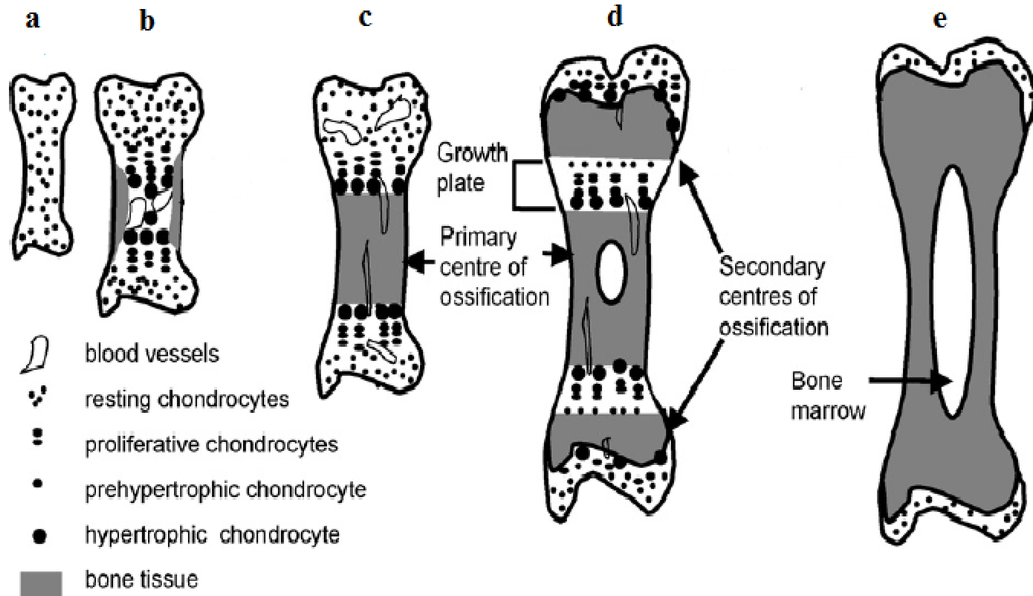

Fig. 2.6. Bone formation modified from Mackie et al. (2008).

In the hypertrophic region, chondroclasts resorb the ECM, while hypertrophic chondrocytes secrete alkaline phosphatase which initiates bone mineral crystal (calcium phosphate, which resembles hydroxyapatite) formation. Chondrocytes then undergo apoptosis and are resorbed. Osteoblasts (bone-forming cells) develop from precursors found in the marrow, and produce the bone matrix from fibrils of type I collagen. Bone mineral crystals grow around this matrix (Whitehead 2004). This process produces a ring of bone in the cartilage at the primary ossification centre which expands towards the ends of the bone. Ossification then begins at the secondary ossification centres located near the bone ends (Fig. 2.6d), moving back towards the centre of the bone (Sjaastad et al. 2003).

Osteoclasts are active in the hypertrophic region of the bone as well, and when followed by osteoblastic bone formation, result in bone remodeling. Bone remodeling allows for the formation of trabecular bone, which is a woven bone made from an irregular structure of collagen fibrils. Most of the trabecular network is resorbed during a continued process of chondrocyte proliferation at the head of the growth plate followed

by chondrocyte hypertrophy and bone mineralization at the end, leaving the marrow cavity free from bone (Whitehead 2004, Fig. 2.6e).

Bone widening occurs when spicules of bone combine to form cavities lined with osteoblasts. The osteoblasts secrete lamellar cortical (structural) bone in concentric layers that eventually fill the cavities. Osteoclasts resorb bone on the inner endosteal surface while bone formation occurs on the outer surface, causing bones to widen in a ring that expands outward. Bone widening does not always occur at the same rate. Early in growth, widening happens quickly so that endosteal resorption begins before the cavities fill with bone. Widening slows as the hen matures, allowing infilling to occur (Whitehead 2004).

2.6.2 Bone Formation During the Laying Period

When hens become sexually mature, the function of the osteoblasts changes from laying down cortical bone to making a type of woven bone, unique to avian and crocodilian species called medullary bone. This change occurs because estrogen stimulates osteoblast function and inhibits osteoclast function (Dacke et al 1993). The increased level of circulating estrogen at the onset of sexual maturity stimulates osteoblasts to lay down medullary bone instead of structural bone. Medullary bone is formed in the spicules of the medullary cavities and on the surface of structural bones, especially the leg bones, and provides a source of Ca for egg shell formation (Whitehead 2004).

The amount of medullary bone increases quickly at the onset of lay and slows down over the laying period (Whitehead 2004). There is almost a complete switch from structural bone production to medullary bone production, but osteoclasts are still active resulting in a net decline of structural bone content (Hudson et al. 1993). During this time, medullary bone is constantly being synthesized, to store Ca, or resorbed, to make Ca available for egg-shell calcification. Because of this constant resorption and synthesis, the total amount of bone may remain unchanged over the laying period (Candlish 1971).

While the total bone content may not change, bone strength may decrease during the laying period because medullary bone is not as strong as structural bone. This is because the majority of medullary bone is located on unconnected spicules and because it

is woven from unusual collagen fibrils. Medullary bone does have some structural properties when there is a decline in cortical bone including maintaining trabecular connectivity and decreasing likelihood of fracture when present in the marrow cavity. Overall however, a bone with a higher medullary content than cortical content will be weaker than a bone with a greater cortical content (Whitehead 2004).

Hens that lay eggs in clutches will put down a new layer of structural bone on top of the layer of medullary bone coating the cortical bone surface, when they stop the egg-laying cycle to incubate the eggs. This allows them to build up a new layer of structural bone, and helps to maintain good bone quality throughout their lives. Commercial hens have been selected to remain in the period of lay which does not give them a chance to build this new layer of structural bone, putting them at greater risk of bone fracture and osteoporosis (Whitehead 2004).

2.6.3 Bone Mobilization and the Calcium Pool

The function of medullary bone is to supply Ca^{2+} for egg shell formation. For this reason, medullary bone has a high turnover rate when compared to other types of bone, during the egg-laying cycle (Etches 1987). Shell formation usually occurs during the dark period, when there is not an adequate supply of Ca from the diet. The increased demand for calcium ions (Ca^{2+}) during shell formation means the hen must get the Ca from another source (Whitehead 2004).

Bone mobilization occurs when the Ca^{2+} concentration of the ECM (ECM [Ca^{2+}]) becomes unusually low (Sjaastad et al. 2003). This results in an increase of osteoclast activity (Whitehead 2004) in areas where mineralization is actively occurring (Sjaastad et al. 2003). Bone tissue is resorbed, releasing Ca^{2+} and P into the ECM where they are available for bone formation in other areas of the skeleton or for egg shell production. The total amount of Ca available to be resorbed and utilized in another way is called the readily-exchangeable calcium pool (Sjaastad et al. 2003). The problem with the increase in osteoclast activity is that these cells do not target a specific type of bone, meaning that any exposed structural bone will be resorbed along with the medullary bone. This leads to an increased risk of osteoporosis because the structural bone is not replaced (Whitehead 2004).

There are several factors that work together to maintain the ECM [Ca^{2+}] between 1.00 and 1.25 millimoles per litre (mmol/L). These factors include Vit D, parathyroid hormone, and calcitonin. Vit D is important because it is the precursor to the hormone calcitrol which is responsible for inducing formation of Ca-binding proteins. These proteins allow transport of Ca from the GI tract into the blood, and from the blood into the shell gland. Calcitrol is necessary for the normal function of osteoblasts and osteoclasts (Sjaastad et al. 2003). Castillo et al. (1979) found that blood plasma concentrations of Vit D (1,25-dihydroxyvitamin D_3) reach a peak just before egg shell calcification, and maintain this level throughout the shell calcification phase of egg laying. A Vit D deficiency will decease blood calcitrol, causing improper mineralization of bone matrix and egg shell (Sjaastad et al. 2003).

Parathyroid hormone (PTH) is produced from two pairs of glands. The first pair is found in the interior of the thyroid glands, and the second is located in the upper section of the thymus. Release of PTH is regulated by a direct negative feedback loop, controlled by the ECM [Ca^{2+}]. When the ECM concentration of Ca^{2+} is within the normal range, PTH is secreted at a constant, moderate rate. This is necessary because PTH has a half-life of only 10 minutes in the blood, making constant secretion necessary for proper Ca^{2+} regulation. The rate of PTH secretion increases when ECM [Ca^{2+}] decreases (de Bernard et al. 1980), with the maximum amount of PTH being secreted when the ECM [Ca^{2+}] is less than 0.7 mmol/L. Similarly, PTH secretion decreases when ECM [Ca^{2+}] increases, reaching a minimum secretion rate when ECM [Ca^{2+}] is greater than 1.25 mmol/L (Sjaastad et al. 2003).

PTH stimulates osteocytes (osteoblasts trapped in the mineralized bone tissue) to increase the release of Ca and P from the stores in the bone (Sjaastad et al. 2003). This was demonstrated by injecting PTH extract into laying hens. After 4 hours there was a significant decrease in bone calcified tissue (Taylor and Belanger 1969). PTH stimulates increased reabsorption of Ca^{2+} and inhibits reabsorption of phosphate by kidneys to maintain a favourable Ca : P ratio. Too much P in the plasma would cause a decrease in the dissolution of bone mineral crystals (Sjaastad et al. 2003).

High plasma concentrations of calcitrol inhibit the synthesis and secretion of PTH. This mechanism is used to control the minimum and maximum levels of PTH

secreted at a given ECM concentration of Ca^{2+}. The concentration of magnesium ions in the ECM also stimulates the release of PTH, but the signal is much weaker than that of Ca^{2+} (Sjaastad et al. 2003).

Calcitonin is a hormone produced by the thyroid gland that reduces the ECM [Ca^{2+}] by inhibiting bone resorption and stimulating urinary Ca excretion. Calcitonin is only secreted in response to Ca when the plasma [Ca^{2+}] rise above 0.9 mmol/L, but can also be stimulated by GI enzymes such as secretin and gastrin regardless of Ca^{2+} concentrations (Sjaastad et al. 2003).

The primary mode of action for calcitonin is to reduce the number of functional osteoclasts on the surface of the bone. The ruffled borders of the cells shrink and lose contact with the bone, reducing the amount of remodelling occurring, and ultimately reducing the amount of Ca and P being released into the plasma (Kallio et al. 1972 and Holtrop et al. 1974).

Ca and P metabolism is controlled by PTH, calcitonin and calcitriol, with the goal of maintaining about 1.2 mmol/L Ca^{2+} and between 0.7 and 1.5 mmol/L P in the ECM. This is done by regulating absorption of dietary minerals from the small intestine, regulating the urinary excretion of these minerals, and regulating release of these minerals from bone tissue. Regulation of the calcium-phosphorus balance is important, because while small Ca^{2+} changes can be adjusted in few hours, several months may be needed to rebuild an exhausted Ca pool (Sjaastad et al. 2003).

2.6.4 Bone Quality Measurements

Bone quality is really referring to bone integrity in terms of bone mineralization, the spacial distribution of the minerals and resistance to fracture. These things together determine the bone strength which is defined by Rath et al. (2000) as toughness or ability to endure stress. Quality is related to the physical (shape, weight, length and width), structural (collagen fibre alignment), and textile (matrix base units) properties of the bone (Rath et al. 2000). There are several methods which can be used to assess bone quality including bone density (bone mineral density, BMD), bone mineral content (BMC), and bone breaking strength (BBS). Many of the methods used to determine bone quality are destructive tests, and can therefore only be measured once per bone.

Bone density can be determined two ways. The first way is to find the bone volume (mL) and the weight of the bone ash (mg) and calculate density (mg ash/mL. The second way is by using quantitative computed tomography (QCT). When using bone volume to calculate density, bone volume can be found by displacement which involves weighing the bone in air and water, and assuming the volume is the same as the weight of the water being displaced by the bone. It is also assumed is that the SG of the water at room temperature (22 °C) is 1.0 g/cm^3 (Zhang and Coon 1997).

The major problems with this method are that it is destructive (because ashing is required) and involves several assumptions. The results can change if even one of these assumptions is changed. For example, the SG of water will be slightly different at a different temperature, meaning the calculations for volume would not be the same if the temperature is 22 °C or 4 °C, and the temperature difference is not taken into account. For this reason, it may be more accurate to find BMD using QCT.

QCT uses an x-ray to take radiographic pictures of a small section of a bone from many angles. This is different from a normal x-ray which measures the attenuation (the difference between radiation emitted from a source and that received by a detector) of the bone, and displays it on a two-dimensional film. The density of the image is calculated using the attenuation, but the same attenuation can be achieved by a small amount of a radio-dense substance or by a large amount of a less radio-dense substance, making it impossible to properly observe the density of one area of interest. QCT accounts for beam width, allowing images to be taken of a three-dimensional voxel (an area of interest with known dimensions). This ultimately allows for the resolution of high and low density objects, even when they are located in close proximity to one another. QCT does not measure the density of the entire bone, but rather measures the density of several small, pre-determined cross-sections along the length of the bone (Korver et al. 2004).

Bone mineral content can also be determined by ashing or QCT. Ashing is done using defatted, dry bones, cleaned of all tissue. An ashing furnace is used, and usually runs overnight at a temperature of 600 °C. The resulting material contains the minerals from the bone sample, so the total mineral content can be determined by comparing the weight of the ash to the original sample weight. The content of specific minerals, Ca for example, can be determined using an atomic absorption spectrophotometry assay

(Cransberg et al. 2001). Again, the major problem with this method is that it is destructive.

QCT can distinguish between cortical bone and bone in the trabecular space (which includes both medullary and trabecular bone) based on the density of each type of bone. The cross-sectional image taken by QCT can therefore be used to determine the area and density of total, trabecular-medullary, and cortical bone. These measurements can then be used to calculate the BMC (mg/mm) of total, cortical and trabecular-medullary bone present in each scan (Jendral et al. 2008).

There are some disadvantages to using QCT as a measure of BMD and BMC. The major disadvantage is the amount of time required to complete one full scan, which can range from 15 to 20 minutes. This includes positioning the bone in the scanner, making the initial scan, selecting the position of the cross-sectional scan, running the cross-sectional scan, and calculating the density from this scan. Additional cross-sectional scans require extra time (around 5 minutes per scan) and for this reason, cross-sectional scans are usually limited to one per bone. With only one cross-sectional scan per bone, conclusions cannot be drawn for BMD along the length of the entire bone. This method still allows for comparison between bones providing all cross-sections are taken at the same place (ratio from a specific end) for each bone (Korver et al. 2004).

Inclusion of raw, ground, full fat canola up to 20 % in broiler diets resulted in no significant effects on mineral (Ca and P) retention, bone ash, or bone Ca and P content (Leeson et al. 1987a).

A study by Gordon and Roland (1997) found that feeding low levels (0.1 %) of NPP resulted in reduced BMC and BMD. However, supplementing the diet with 300 U/Kg of phytase significantly improved both the BMC and BMD. When higher levels of NPP were fed, there was no change in bone quality factors, even when supplemental phytase was added.

Bone breaking strength is one factor that gives information about bone fragility. The other two factors are brittleness and work-to-failure. All three of these factors can be measured using biomechanical tests which load the bone with force until it breaks. The breaking force is recorded, and a force-displacement curve is generated. The height of the curve is bone strength (ultimate force) in newtons (N) or kg force (Turner 2002).

Bone breaking strength is often measured using a three-point compression test, which requires a stage with fixed points that can support the bone. The distance between the two fixed points should be adjustable so that different types of bone (tibia or humerus, for example) can be tested. A blade used as the third compression point, travels at a known rate, and contacts the bone at the midpoint, applying pressure until the bone breaks. The force required to break the bone is recorded using software. The bones should be positioned on the stage so that the blade strikes all bones on the same facial plane, allowing comparisons to be made between bones. An example of BBS measured in this manner is outlined by Fleming et al. (1994).

A study by Riczu et al. (2004) compared bone quality measurements of brown-shelled layers and white-shelled layers using the femur and the humerus. They found that brown-shelled layers had a lower femur trabecular density than white-shelled layers, but had higher femur total and cortical area, femur BBS (in Kg force) and bone weight (in g) than the white-shelled layers. As well, the brown-shelled layers had higher humerus total and cortical density, cortical area, BBS, bone weight, and bone length than the white-layers. Some of these measurements are expected (bone weight, length, and maybe breaking strength) because the brown-shell laying hens are larger than the whites. These differences suggested that the brown-shelled strain of hen was able to mobilize more Ca from medullary bone, allowing preservation of the cortical (structural) bone as suggested by the lower trabecular density (which includes medullary bone content) and higher cortical area and breaking strength of the brown-shelled hens (Riczu et al. 2004).

Very little work has been done to assess the effects of CM on bone quality. A study using broiler chickens found that feeding a cold-pressed yellow-seeded RSM resulted in a greater tibia weight and breaking strength than feeding a cold-pressed black-seeded RSM. However, the study also found that feeding two other yellow-seeded RSMs were not different from the black-seeded meal (Czerwiński et al. 2012). No work has been reported using CM or JM for laying hens in respect to bone quality measurements.

Because bone (especially medullary bone) is used as a source of minerals for egg shell formation, bone quality measurements can be correlated with egg production and egg quality measurements. For example, one study found that femur trabecular area was positively correlated with egg SG and weight, while humerus and femur total area were

correlated with body weight (Riczu et al. 2004). Another study found that measurements of humerus and tibiotarsus breaking strength were not correlated with egg breaking strength (Hocking et al. 2003). This indicates that not all bone and egg quality measures will be affected by mobilization for shell formation.

2.7 Liver Health

The liver of the laying hen has many important functions. Along with bile production, which is required for the breakdown and absorption of fat from the intestine, the liver is responsible for regulating nutrient release into the blood and regulating excretion, through the bile, of exogenous and endogenous substances. The liver is involved in the production of blood coagulation factors and plasma proteins, production of cholesterol, and the inactivation or conversion (to a water-soluble form, making excretion through urine easier) of hormones, drugs, and toxins. All of these roles are required to keep the body functioning normally, and liver damage can result in abnormal absorption of fat, abnormal digestion, bleeding disorders, and increased activity of hormones or a prolonged action of some drugs (Sjaastad et al. 2003).

Rapeseed meal has been shown to cause liver damage in laying hens and broiler chickens in the past. Marangos and Hill (1976) found that feeding 12 % *Brassica napus* RSM to laying hens caused an increased liver weight compared to the *Brassica campestris* or SBM control groups. As well, *B. napus* fed hens had an increased mortality rate over hens fed *B. campestris* or a SBM control diet. In the final 4 week period of the trial there were 15 mortalities, 12 of which were sent for necropsy. The cause of death for 10 of the 12 hens was found to be massive liver hemorrhage. All 10 of these hens were consuming a RSM diet, either *B. napus* or *B. campestris*. This study determined that hens in full production seem to be more susceptible to liver hemorrhage when fed RSM than younger (growing) birds.

2.7.1 Lipid Metabolism

The majority of FA synthesis occurs in the liver of birds, not in the adipose tissue (Griffin et al. 1992), as it does in mammals, which causes the metabolic activity of the liver to be particularly high in poultry (Butler 1976).

The metabolic activity is further heightened during egg production because ovarian hormones (estrogen) stimulate lipogenesis. In fact estrogenized male broilers showed an increase in plasma very low density lipoprotein concentrations (from 0.158 mg/ml to 40.4 mg/ml) over the control group (Hermier et al. 1996).

Each egg formed requires more than half of the lipid content of the liver, ensuring that the liver always had to work to replace it. In fact, in a 1976 review of the literature, Butler reported that for each year of egg production the hen has to synthesise its own body weight in lipid. This would be even more dramatic today, as hens lay an increased number of eggs per production cycle compared to hens in 1976.

There are two main pathways for lipid metabolism in the laying hen. The first (simplest) pathway begins in the small intestine of the laying hen, where lipases partially hydrolyse dietary fat. Bile salts produced in the liver enter the lumen of the intestine and form micelles with the fat. The micelles then pass into the mucosal cells, where lipid re-synthesis occurs. A majority of the fat then enters the blood stream as LDL (Butler 1976).

When the diet does not contain much fat, the laying hen must have another way to produce the large amounts of lipid required for each egg yolk. This is done through a complex pathway that allows carbohydrates to be converted into lipids (Fig. 2.7).

The pathway begins with the ingestion of carbohydrates, which are broken into their simple sugars (glucose) through the process of digestion. The reaction begins in the cytosol of the cell, moves into the mitochondria, and then back into the cytosol, where the fatty acids are actually produced from acyl-Co A derivatives. The most important rate limiting enzyme in saturated FA synthesis is Acetyl-Co A carboxylase because it is activated by carbohydrate intake (increased citrate), and inhibited by lipid intake or synthesis (acyl-Co A derivatives) (Butler 1976).

Once saturated FA have been formed, they can be desaturated to become unsaturated FA such as oleic or palmitoleic acid (both non-essential FA). The acyl-Co A derivatives can also be made into glycerides, phospholipids, and cholesterol esters (Butler 1976).

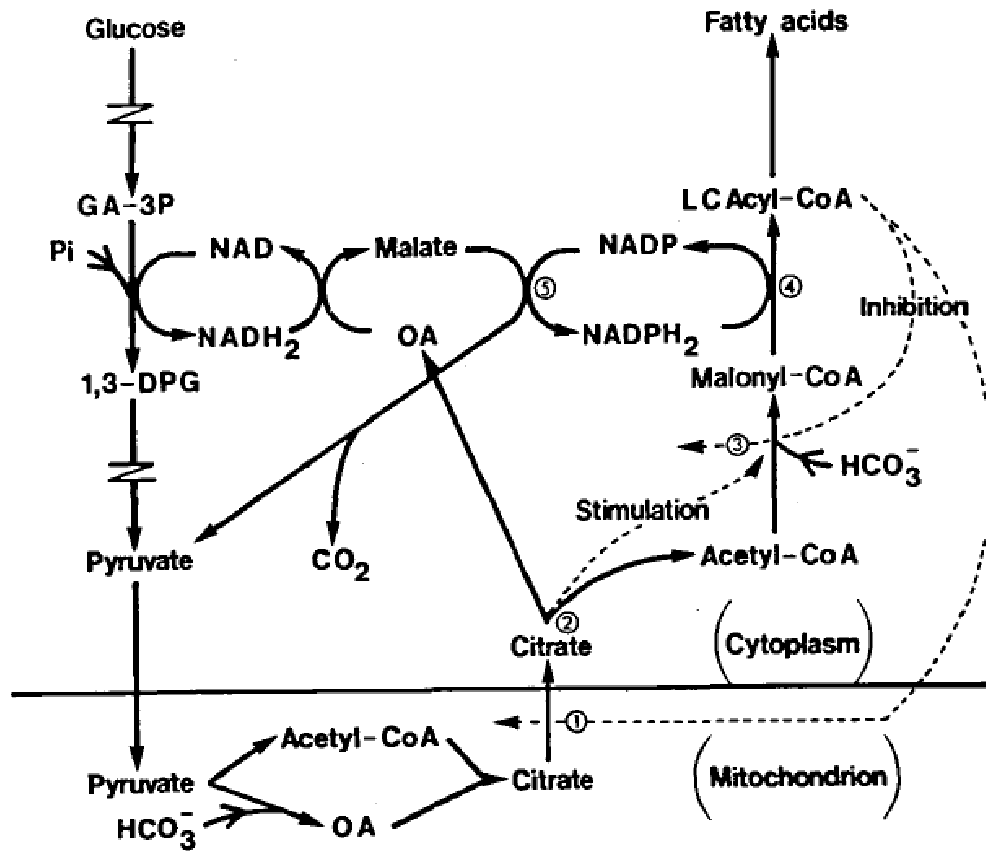

Fig. 2.7. Saturated fatty acid synthesis from carbohydrate from Butler (1976).

The pathway used a series of lipogenic enzymes which are indicated by the numbers 1 through 5. (1) citrate translocation system, (2) ATP citrate lyase, (3) acetyl-Co A carboxylase, (4) fatty acid synthetase, and (5) 'malic' enzyme.

While adipose tissue in the laying hen does not synthesise the majority of the lipid found in the body, it does have other functions. These functions include lipid storage, generating glycerol, and integrating the FAs produced by the liver into glycerides (Leveille et al. 1975). Lipids arrive at the adipose tissue, in the form of low density lipoproteins, where they are liberated from the protein and hydrolysed by an enzyme found in the capillary wall. Finally the lipids are esterified with α-glycerophosphate, which is derived from glucose, and are stored as triglycerides until they are needed (Leveille et al. 1975).

2.7.2 Hepatic Steatosis

Hepatic steatosis (fatty liver) can be caused by several factors including intensified lipogenesis, a decrease in lipid transport away from the liver, a decline in lipid deposition in the adipose tissue, and a reduction in lipid oxidation. Lipogenesis can be increased when there is high amount of carbohydrate in the diet due to the fact that lipogenesis is linked to glycolysis (Butler 1976).

Lipid transport can be reduced if the diet does not contain sufficient amounts of amino acids required for apolipoprotein synthesis, or if reduced by a deficiency of phospholipid, which can be caused by inadequate amounts nutrients involved in the synthesis of choline (vitamin B_{12}, folic acid and methionine) or a choline or inositol deficiency. Apolipoprotein and phospholipid are both required for lipoprotein synthesis, so the absence of either would cause a build-up of lipid in the liver (Butler 1976).

Decreased lipid deposition in the adipose tissue may be responsible for lipid build-up in the liver tissue, but more research needs to be done in this area to confirm this. The probable mode of action is an inhibition of the enzyme lipoprotein lipase, which controls the deposition of lipid into the adipose tissue. Stress to the hen can accelerate the production of cyclic AMP which inhibits lipoprotein lipase, but also activates the lipase system in the adipose cells which hydrolyse lipid so it can be released into circulation. With the inhibition of lipoprotein lipase and the activation of the lipase system, the hen can become hyperlipidemic, which may lead to the development of a fatty liver (Butler 1976).

A reduction of lipid catabolism in the liver can also cause a fatty liver. Lipid oxidation involves several systems including the β-oxidation system, the Krebs cycle, and the respiratory chain. All of these systems require some vitamin (eg. riboflavin) or trace element (eg. iron) as a co-factor, and for this reason, the catabolism of lipids can be inhibited at several points, and because of any number of nutrient deficiencies (Butler 1976).

As well Hermier et al. (1996) showed that male broiler chickens given an intramuscular injection of estrogen (20 mg/kg of 17β-estradiol in propylene glycol) had liver weights 2 times greater, and liver lipid contents (g/liver) 8 times greater than the

control group. This may indicate that livers of laying hens are naturally fatty once lay starts, pre-disposing them to other liver conditions and diseases.

2.7.3 Fatty Liver Hemorrhagic Syndrome

Fatty liver hemorrhagic syndrome is a disease in laying hens that often has no symptoms until sudden death of a hen occurs. There may be some outward signs which include pale, enlarged combs, wattles covered with 'dandruff' (Harms et al. 1972), an abrupt drop in egg production, and undue nervousness. Upon necropsy it becomes apparent that death was caused by a massive hemorrhage of the liver. This hemorrhage ruptures the Gilsson's capsule (which surrounds the liver) causing blood to flow into the abdominal cavity (Butler 1976). The liver itself is enlarged, pale yellowish-brown, has indications of smaller hemorrhages that did not rupture the Glisson's capsule, and is extremely friable. The hen may also have enlarged, pale kidneys and a great deal of yellow abdominal fat in the body cavity and around the viscera. Necropsy of other hens in the flock often show livers with small hemorrhages under the Glisson's capsule and large amount of abdominal fat (Fowler 1990).

Histological examination of the liver shows that the majority of hepatocytes are extremely swollen with fat (Martland et al. 1984), while others have ruptured (Butler 1976). The structure of individual hepatocytes is often disorganised because fat accumulates as globules in the vacuoles of the cytoplasm. This pushes the cell nucleus off to the side of the cell, and can cause it to degenerate (Butler 1976). In many cases, there is also damage to the reticulin bands which provide structural support to the cell. The overall cellular structure of the liver is also disrupted by capillary hemorrhages, blood clots, vascular breakdown, and zones of fibrosis and necrosis. All of this results in structural weakness of the liver, causing friability (Martland et al. 1984).

A study by Cherian et al. (2002) found that feeding 0.5 % of conjugated linoleic acid (CLA) to laying hens caused pale livers with a fat content two times greater than the control hens. Increasing the level of CLA in the diet to 2.0 % caused extensive fat deposition and greater than 75 % cytoplasmic vacuolation in a majority of cells. As well,

large liver hemorrhages were noted upon necropsy in some of the hens consuming 2.0 % CLA.

Analysis of the liver from FLHS hens for fat content found levels that ranged from 40 % to as high as 70 % fat, on a dry weight basis (Fowler 1990). Lipid analysis showed that the concentrations of the existing FAs had changed, with higher concentrations of oleic acid, but no new FAs were incorporated into the lipid. Analysis was also performed on blood plasma, which showed lipid levels echoed those found in the liver (Butler 1976).

In past studies, RSM has been shown to cause (Gough and Weber 1978) or modify (Pearson et al. 1978b) the symptoms of FLHS in laying hens. Although not all cases were diagnosed as FLHS (as with the study by Gough and Weber 1978), liver hemorrhages and mortalities were linked to the inclusion of RSM in the diets.

2.7.4 Liver Health Measurements

RSM had been known to cause physiological changes to the liver therefore, it is important to evaluate the liver to determine whether CM or JM cause these changes as well. There are many ways in which the liver can be evaluated to determine if any changes or damage has occurred. These include weight, chemical composition (dry matter, fat content), tissue colour, tissue texture, and the presence of lesions. All of these methods require the euthanasia of the hen before measurements can be taken.

Liver weight is an easy measure of quality that can be used as a first sign that some change or damage has occurred. For example Akiba et al. (1983) found that anti-thyroid compounds administered to laying hens caused an increase in thyroid weight, and an increase in both liver weight and liver lipid content. This study showed that liver weight could be an indicator of liver lipid content, but may also be able to indicate changes occurring in less easily measured tissues such as the thyroid gland.

Hepatosomatic index (HSI) is another way to measure liver weight, but this measure expresses the weight of the liver in terms of the hen body weight. This measure would allow for comparison of liver weights among hens because it accounts for the fact that a larger hen would have a larger liver than a hen of a smaller body weight. HSI is used as a measure of liver weight in many areas of research including mink (Rouvinen-

Watt et al. 2010), fish (Lanari et al. 1998 and Yang et al. 2011), quail (Brausch et al. 2010), broiler chickens (Kermanshahi and Abbasi Pour 2006, Smulikowska et al. 2006 and Ebrahimnezhad et al. 2008) and laying hens (Cheva-Isarakul et al. 2001 and Han et al. 2010).

HSI for white- and brown-shelled laying hens was compared by Riczu et al. (2004). White-shelled layers had a greater HSI than brown-shelled layers, but also had higher egg production. Greater HSI could be explained by the increased lipid production in the liver which is necessary to sustain a higher rate of egg production.

Chemical composition of the liver includes dry matter and fat content, which can be used to confirm whether physiological changes have taken place in the liver. DM is determined by taking the wet weight of the liver, freeze-drying the sample to remove all moisture, and then weighing the sample again (Thomas et al. 1978).

Measuring fat content gives information about the total amount of lipid found in liver tissue. It does not give information about lipid composition, or amounts of specific FAs. Fat content is measured on the freeze-dried samples, and results are presented on a DM basis. Fat content can be measured in several ways, and with several solvents. To determine if one method of fat extraction was more accurate than another, Dobush et al. (1985) tested samples of ground snow goose carcass using a Soxhlet or Goldfisch extractor with four solvents (petroleum ether, diethyl ether, chloroform-methanol, and a mixture of petroleum ether and chloroform-methanol) over four time periods (3, 6, 12, and 24 hours). For determining body composition of birds, extraction with petroleum ether or diethyl ether as the solvent gave the more accurate results. This was because after 6 hours of extraction, the amount of lipid being extracted from a 5 g sample had leveled off with these solvents, and they extracted significantly less non-lipid material than the chloroform-containing solvent.

Liver scoring is a method which can be used to determine the health status of a liver. A healthy liver should be dark reddish-brown in colour, firm in texture and should be free of visible lesions such as hemorrhages. A damaged liver may be pale in colour, friable in texture and may contain hemorrhages. Colour can be measured using a visual scoring system or a colourimeter, which will give less subjective results. A colourimeter is an instrument that measures colour over a range of visible wavelengths (approximately

400 – 700 nm) which correlate with the human brain-eye perception. It works by isolating a broad band of wavelengths using a tristimulus absorption filter, located at the sensor. The simple data processor in the instrument takes these readings and displays them as tristimulus values (HunterLab 2008a). In this case, tristimulus values are 'L' which measures light to dark, 'a' which measures green to red, and 'b' which measures blue to yellow (HunterLab 2008b).

There are several different colour scales which can be used to report colour. One of these is the Hunter L, a, b scale and another is the CIELAB scale (L*, a* and b*). Both are based on Opponent-Colour Theory, which assumes that the human eye recognizes colour as three opposite pairs: light-dark, red-green and yellow-blue. The difference between the two scales is how the value for L (L*), a (a*) or b (b*) are calculated from the values initially read by the colourimeter. The CIELAB scale is an updated version of the Hunter L, a, b scale, but both are acceptable when reporting colour values (HunterLab 2008c).

Liver colour in broiler chickens has been measured using a colourimeter, but not with regard to use of CM, JM or dietary enzymes. Northcutt et al. (1997) measured the relationship between feed withdrawal and viscera condition of broilers and was successfully able to determine a difference in redness ('a') and yellowness ('b'), but lightness ('L') was not affected.

A study by Trampel et al. (2005) found that L* (lightness) values had a strong positive correlation with the concentration (% of liver weight) and the quantity (g) of lipids in the liver tissue. They also found that a* (redness) values had a strong negative correlation with the concentration and quantity of liver lipids. These results indicate that a liver that is lighter (higher L* score) and less red (lower a* score) would have a higher amount of liver lipids.

Colour can also be evaluated using a visual scoring system. These systems allow the observer to rank the colour of the liver from dark (red) to pale (yellowish-brown) in a series of increments. A study measuring the effect of stunning method on the liver quality of geese devised a scoring system similar to that used to evaluate meat quality where 'light' coloured livers received a score of 1, 'normal' coloured livers received a score of 2, and 'dark' coloured livers received a score of 3 (Turcsán et al. 2001).

Texture scoring of livers is used in the 'foie gras' industry to ensure that livers have the desired texture to be used as raw, whole fatty liver products. A study by Fernandez et al. (2010) used texture scoring as part of the 'foie gras' industry grading system. In the 'foie gras' industry 'excessive firmness' is considered a downgrade because it indicates the desired level of fat infiltration had not been reached (Fernandez et al. 2011). In laying hens however, a firm liver is desired. Wolford and Polin (1972) used liver texture scoring in a study of FLHS in laying hens. Livers were classed as firm, less firm, or ruptured easily. The study found that livers of hens diagnosed with FLHS ruptured more easily than livers from hens with FLHS (Wolford and Polin 1972).

Hemorrhage scoring is a method of visual scoring that helps to determine whether a hen has FLHS. Several different hemorrhage scoring systems have been used to evaluate livers of poultry including basic systems like the one by Turcsán et al. (2001) where livers received a 1 if there was no hemorrhaging, a 2 if hemorrhaging was mild, or a 3, if severe hemorrhages were present. A more descriptive system could include types and amounts of hemorrhages, like the one by Diaz et al. (1999) where livers were scored from zero to 3, where 0 indicated no hemorrhages, 1 indicated 10 or less subcapsular petechial or ecchymotic hemorrhages, 2 indicated greater than 10 subcapsular petechial or ecchymotic hemorrhages, and 3 indicated a massive hemorrhage which ruptured the Glisson's capsule.

Hemorrhage score had been used in several studies with laying hens where flaxseed (Schumann et al. 2003 and Leeson et al. 2007) or RSM (Leeson et al. 1976 and Leeson et al. 1978) were fed, but has not been evaluated when canola or juncea meals were fed to laying hens.

2.8 Current Recommendations on Maximum Levels of Canola and Juncea Meals for Use in Laying Hen Diets

2.8.1 Recommendations on Canola Meal for Use in Laying Hen Diets

According to the Canola Council of Canada (2011), the current recommended maximum level of canola meal that should be included in laying hen diets is 10 %. This is based on animal health concerns and ration formulation techniques, but the main reason

given for this maximum inclusion level is that there are potential effects on mortality (Newkirk 2009).

During the 1970's many studies were done with rapeseed meal (the precursor to canola meal) that found increased mortality (Marangos et al. 1974 and Olomu et al. 1975), decreased egg production (Marangos et al. 1974, Olomu et al. 1975, Leeson et al. 1976 and Marangos and Hill 1976) and decreased egg weight (Marangos and Hill 1976) when RSM was included at 10 % or more of the diet. During this time, breeding work was being done in Canada with RSM to reduce the glucosinolate content (Stefansson and Kondra 1975). This resulted in the development of rapeseed meals like the variety Tower, which had a lower incidence of mortality due to liver hemorrhage, and higher egg production than RSM being used in Britain at the same time (Ibrahim and Hill 1980).

In the late 1980's studies began to appear focusing on CM for broiler chickens and laying hens. One study with broiler chickens found that CM could be substituted for a portion of SBM without causing a deficiency in minerals such as chloride, copper, iron, magnesium, manganese, or zinc (Summers and Leeson 1985). When CM was investigated for laying hens it was found that substituting 10 % CM into a corn-SBM based diet, on an isocaloric basis, caused a reduction in feed consumption, egg production and egg weight (Summers et al. 1985 and Summer et al. 1987) similar to what was found with RSM in the 1970's. However, another study found that it was possible to completely replace SBM with CM (resulting in 25 % CM to be included in the ration) with no negative effects on production performance, nutrient retention or bone mineralization (Leeson et al. 1987b).

After this period of work with CM in the 1980's, not much work was done until the late 1990's or early 2000's, when use of full-fat canola seed (Talebali and Farzinpour 2005), as well as the use of dietary enzymes such as a multicarbohydrase (Simbaya et al. 1996) and/or phytase (Leske and Coon 1999) began to be investigated. Most of this work was done with broiler chickens, and although enzymes have been investigated for use in laying hen diets (Jalal and Scheideler 2001, Lázaro et al. 2003 and Silversides and Hruby 2009), little or no work exists on using these enzymes in combination with CM for layers.

It appears that the current recommended maximum levels of CM for use in laying hen diets were set based on the work completed before 1990. With the improvements that

have been made to canola since the late 1980's (such as a further reduction in total glucosinolates) and enzyme use, it may be possible to include CM at levels higher than 10 %, without the effects on mortality and egg production that have been seen in the past.

2.8.2 Recommendations on Juncea Meal for Use in Laying Hen Diets

In the 1970's some work was done with mustard meal (*Brassica juncea*) for laying hens. One study found that feeding 12 % juncea meal caused a decrease in egg production and egg weight, as well as a significant number of mortalities due to liver hemorrhage (Marangos et al. 1974). Another study found that feeding 12 % juncea meal did not affect hen performance, but did cause an increase in thyroid weight (Marangos and Hill 1976). Both the increase in mortality and the increase in thyroid weight were attributed to high levels of GLS in the meals.

In 1990, Love et al developed a low glucosinolate variety of *Brassica juncea*. It did not meet all of the requirements to allow it to be called canola-quality at this time. However, in 1999, Potts et al. developed a variety of juncea that met the canola-quality standards.

Since the development of canola-quality juncea meal, little work has been done with laying hens. One study found that up to 10 % mustard meal could be included in diets for laying hens. As well, 20 % could be used without detrimental effects on production but hen health was adversely affected at this level. This study did not indicate whether the variety of *Brassica juncea* used in this experiment was of canola-quality (Cheva-Isarakul et al. 2001).

The Canola Council of Canada does not currently list recommendations for the maximum level of juncea that can be included in diets for laying hens. This may be because not enough research has been done with mustard seed or meal for poultry, especially canola-quality mustard. The consensus in the literature seems to be that JM should not be included in diets for laying hens at more than 10 %.

Dietary enzymes could be used to improve the feeding value of JM. No research has been done with JM to determine the effects of a multicarbohydrase or phytase enzyme for use in laying hen diets, but some work has been done with broiler chickens.

As summarized in a review of the literature, the inclusion of a multicarbohydrase enzyme in broiler diets containing JM resulted in increased energy (AME) utilization (Khajali and Slominski 2012). More work still needs to be completed in this area.

Similar to CM, advances have been made in plant breeding which have further reduced the glucosinolate content of JM. With these improvements and the addition of dietary enzymes, it may be possible to include JM in diets for laying hens at levels greater than 10 % without negative effects to performance and hen health that were found in previous studies.

2.9 Focus of Literature

With an increased interest in converting oil from canola into biofuel, there is growing availability of meals with oil contents ranging from 1-3 % when extracted using pre-pressed solvent-extraction. Breeding has been successful in reducing the erucic acid level in the oil and the glucosinolate content in the meal. This results in a decrease in anti-nutritional factors, potentially allowing higher inclusion levels of canola meal, with no negative effects on bird performance or health.

Similar to the selection and breeding in canola, developments are also being made with juncea which allow it to meet the canola quality standards. The decrease in anti-nutritional factors led to the evaluation of juncea for broiler chickens but it still needs to be evaluated for use in laying hen diets in terms of hen performance and health.

Carbohydrases and phytases have been evaluated for broilers in diets containing CM, but work needs to be done with laying hens to determine the ability of enzymes to improve laying hen performance and health when diets contain CM. Carbohydrases have been studied in diets containing JM for broiler chickens, but have not yet been evaluated for laying hens. Phytases have not been evaluated in combination with JM for poultry. For these reasons, carbohydrase and phytase need to be studied with CM and JM for laying hens.

This project addressed these questions using a feeding trial. Canola and juncea were included as pre-pressed solvent-extracted meals, at three levels in the diet. This trial assessed juncea as a feedstuff for layers, evaluated improved varieties (reduced

glucosinolate and erucic acid) of canola, and evaluated the addition of carbohydrase and phytase enzymes to improve the feeding quality of both of the meals.

2.10 Objectives

1. To evaluate the effects of pre-pressed, solvent-extracted *Brassica napus* and *Brassica juncea* meals, included in the diet at three levels (0, 10 or 20 %), on the production performance of white and brown strains of laying hens.
2. To compare the effects of the presence or absence of supplemental dietary enzymes (combination of Bio-Phytase and Superzyme OM™) on the production performance of white and brown strains of laying hens.
3. To evaluate the inclusion of canola and juncea meal on egg quality, bone characteristics and liver condition in hens fed these meals for a 48 week period.
4. To compare the effects of supplemental dietary enzymes on egg quality, bone characteristics and liver condition in hens fed these meals for a 48 week period.

Chapter 3. Effect of canola meal or juncea meal with or without supplemental dietary enzymes on production performance and mortality of white- and brown-shell egg laying hens.

3.1 Abstract

Recently developed low-glucosinolate canola (*Brassica napus*) meal (CM) and juncea (*Brassica juncea*) meal (JM) were evaluated in laying hen diets. Two trials designed as 5x2 factorials in completely randomized design compared the effects of CM, JM or soybean meal (SBM), with and without enzyme supplementation, on egg quality characteristics. A total of 360 Lohmann LSL-Lite White (Trial 1, WSLH) or 300 Lohmann Brown-Lite (Trial 2, BSLH) laying hens, housed in 60 cages, were fed one of 10 isoenergetic and isonitrogenous diets (SBM, 10 % CM, 20 % CM, 10 % JM or 20 % JM with or without a dietary enzyme cocktail of Superzyme OM™ and Bio-Phytase™) for 48 weeks. Feed consumption (FC) and hen-day egg production were measured and feed conversion ratio (FCR) was calculated at the end of each 28 day period. Body weights were measured initially and at the end of each period. Abdominal fat pad was weighed at the end of the trial, and converted to a percentage of body weight. Necropsies were performed on any hens that died or were culled throughout the course of the trials. There was an enzyme x period ($P = 0.0011$) effect on FC for BSLH where enzyme supplementation reduced feed consumption in periods 2, 4, 6 and 7. There was a meal x period effect for FC of BSLH where 20% JM fed hens consumed less feed than SBM fed hens (113 versus 106 g/hen/day). Meal had a marginally significant effect on egg production of WSLH ($P = 0.0533$) where 10 % JM resulted in less eggs than 10 % CM fed hens (93.5 versus 90.1 %). There was a meal x period effect ($P = 0.0015$) on body weight of WSLH where in most periods, 20 % JM decreased body weight compared to SBM. Meal decreased fat pad weight as a percentage of body weight for 20 % JM fed WSLH compared to the SBM fed WSLH from 4.25 to 3.20 %. Enzyme supplementation decreased ($P = 0.0440$) body weight for BSLH from 2146 g to 2089 g. There was an enzyme x period effect on FCR for WSLH and BSLH where enzyme supplementation improved FCR in period 5 from 2.01 to 1.93 (WSLH) and from 2.01 to 1.91 (BSLH). Based on these results, up to 20 % CM or JM can be included in diets of white- and brown-shell egg laying hens. Enzyme should be included in diets for WSLH, but is not necessary in diets of BSLH.

Key Words: *Canola, Juncea, Phytase, Mulitcarbohydrase, Production Performance, Poultry*

3.2 Introduction

Rapeseed meal, the precursor to canola meal, and canola meal itself have reduced production performance factors such as feed consumption (Summers et al. 1987), egg production and feed conversion ratio (FCR, Marangos et al. 1974) when fed to laying hens. Similarly, juncea meal (or mustard meal) reduced feed consumption, body weight (Cheva-Isarakul et al. 2001) and FCR (Marangos et al. 1974) when included in laying hen diets.

The anti-nutritional factors in CM and JM could be the cause of the reduction in production performance. Glucosinolates, found in both CM and JM (Newkirk et al. 1997), are hydrolysed into breakdown products which are responsible for bitterness and morphological changes to the thyroid, liver and kidneys. Fibre (Choct 2002) and phytate (Newkirk 2009) can bind minerals and other nutrients making them unavailable to laying hens.

Plant breeding programs have reduced the negative impacts of glucosinolates (and their breakdown products) in canola (Stefansson and Kondra 1975) and juncea meals (Love et al. 1990). Very little recent work (less than 20 years old) has been completed evaluating the effects of these low glucosinolate meals on production performance.

Commercially available multicarbohydrase and phytase enzymes have been used to overcome the effects of fibre and phytate (Boling et al. 2000, Lázaro et al. 2003 and Han et al. 2010) on laying hens, but enzymes need to be evaluated in combination in laying hen diets which contain canola or juncea meal.

3.3 Objectives

1. To determine the effect of canola meal or juncea meal included at 10 or 20 % of the diet on production performance characteristics of white- and brown-shell egg laying hens including: hen body weight, fat pad weight as a percentage of body weight, feed consumption, egg production, feed conversion and mortality.
2. To determine the effect of a supplemental dietary enzyme cocktail on production performance characteristics of white- and brown-shell egg laying hens including: hen body weight, fat pad weight as a percentage of body weight, feed consumption, egg production, feed conversion and mortality.

3.4 Hypothesis

The inclusion of canola or juncea meal will not have a significant effect on production performance characteristics of white- or brown-shell egg laying hens including: hen body weight, fat pad weight as a percentage of body weight, feed consumption, egg production, feed conversion and mortality. The inclusion of dietary enzyme will decrease feed consumption and improve egg production and feed conversion, but will not have a significant impact on body weight, fat pad weight as a percentage of body weight or mortality of white- or brown-shell egg laying hens.

3.5 Materials and Methods

3.5.1 Animals and Husbandry

This project consisted of two 48 week production studies using 360 Lohmann LSL-Lite White (Trial 1) and 300 Lohmann Brown-Lite (Trial 2) laying hens. Hens were 30 weeks of age at the commencement of trials and were randomly assigned to 60 cages per trial (6 white hens or 5 brown hens per cage). The cages were located in the two middle tiers, and the bottom tier on the back of a double-sided three-tier battery cage system. Each cage provided 480 cm^2/hen. A 5x2 factorial in a completely randomized design was used, with the main factors being dietary treatment (soybean meal control, 10 % canola meal, 20 % canola meal, 10 % juncea meal, and 20 % juncea meal) and supplemental enzymes (either present or absent). One of ten dietary treatments was randomly assigned to each cage, giving six replications per treatment. Hens consumed the feed *ad libitum* from feed troughs placed in front of the cages and received water *ad libitum* from nipple drinkers. The hens were provided with 15 hours of light per day at an intensity of 10 lux and were housed in an environmentally controlled room maintained at 22 - 24 °C. All hens that died or were culled during the course of the experiment were necropsied by a veterinary pathologist. All birds were managed in accordance with the Dalhousie Agricultural Campus Animal Care and Use Committee guidelines which follow the Canadian Council on Animal Care Codes of Practice (2009).

3.5.2 Experimental Diets

The 10 dietary treatments (Table 3.1) consisted of a soybean meal control, 10 or 20 % canola meal, and 10 or 20 % juncea meal all with (+E) or without (-E) supplemental dietary enzymes. The canola meal and juncea meal (from seed grown in western Canada) were provided by the Canola Council of Canada. The enzyme cocktail was a combination of the commercially available enzymes, Bio-Phytase and Superzyme-OM™, supplied by Canadian Bio-Systems Inc. Calgary, Alberta. The experimental diets were formulated to be isoenergetic and isonitrogenous with corn, soybean meal, and poultry fat as major ingredients that were allowed to fluctuate. Wheat was included in all diets at 10% as an energy source. The canola meal and juncea meal were added to the diet by substituting for soybean meal. All diets were formulated to meet laying hen nutrient allowances recommended by the breeding company (Lohmann Tierzucht 2007) for each phase of the hen's laying cycle represented in the duration of the study. These breeding company allowances met or exceeded the NRC (1994) nutrient requirements.

Table 3.1. Dietary treatment abbreviations

Diet Name	Abbreviation
Soybean Meal Control	SBM
10 % Canola Meal	10 % CM
20 % Canola Meal	20 % CM
10 % Juncea Meal	10 % JM
20 % Juncea Meal	20 % JM
Soybean Meal Control + Enzyme	SBM + E
10 % Canola Meal + Enzyme	10 % CM + E
20 % Canola Meal + Enzyme	20 % CM + E
10 % Juncea Meal + Enzyme	10 % JM + E
20 % Juncea Meal + Enzyme	20 % JM + E

The feeding period encompassed four phases with diet changes at 42, 50 and 60 weeks of age. Protein requirements decreased as the hens aged, while calcium requirements increased to meet the increasing calcium need of the laying hen. Diets for phase 1 (Table 3.2 and Table 3.3) and 2 (Table 3.4 and Table 3.5) were formulated based on a feed consumption of 105 g/hen/day. Diets for phase 3 (Table 3.6 and Table 3.7) and 4 (Table 3.8 and Table 3.9) were based on a feed consumption of 110 g/hen/day.

Table 3.2. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets without enzyme for phase 1 (30 to 41 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	53.25	50.02	46.70	50.02	46.70
Soybean Meal	25.04	17.31	9.60	17.31	9.60
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	4.96	4.91	4.85	4.91	4.85
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	2.98	2.95	2.93	2.95	2.93
Poultry Fat	1.35	2.52	3.71	2.52	3.71
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.37	0.35	0.33	0.35	0.33
Methionine Premix[3]	0.23	0.14	0.09	0.14	0.09
Iodized Salt	0.32	0.31	0.29	0.31	0.29
Total	100.00	100.01	100.00	100.01	100.00
Calculated Composition (% as fed)					
Protein	16.70	16.70	16.70	16.70	16.70
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	3.60	3.60	3.60	3.60	3.60
Non-Phytate Phosphorus	0.33	0.33	0.33	0.33	0.33
Methionine + Cystine	0.69	0.69	0.71	0.69	0.71
Lysine	0.96	0.92	0.89	0.92	0.89
Determined Composition (% as fed)					
Crude Protein	16.19	18.52	17.47	15.29	15.77
Crude Fat	3.79	5.21	6.04	4.91	6.43
Calcium	4.56	3.55	2.75	3.74	3.90
Total Phosphorus	0.45	0.48	0.53	0.46	0.50
Sodium	0.15	0.13	0.13	0.13	0.15

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal

[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 3.3. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets with enzyme for phase 1 (30 to 41 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	53.25	50.02	46.70	50.02	46.70
Soybean Meal	25.04	17.31	9.60	17.31	9.60
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	4.96	4.91	4.85	4.91	4.85
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	2.98	2.95	2.93	2.95	2.93
Poultry Fat	1.35	2.52	3.71	2.52	3.71
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.37	0.35	0.33	0.35	0.33
Methionine Premix[3]	0.23	0.14	0.09	0.14	0.09
Superzyme OM[4]	0.05	0.05	0.05	0.05	0.05
Biophytase[5]	0.01	0.01	0.01	0.01	0.01
Iodized Salt	0.32	0.31	0.29	0.31	0.29
Total	100.06	100.07	100.06	100.07	100.06
Calculated Composition (% as fed)					
Protein	16.70	16.70	16.70	16.70	16.70
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	3.60	3.60	3.60	3.60	3.60
Non-Phytate Phosphorus	0.33	0.33	0.33	0.33	0.33
Methionine + Cystine	0.69	0.69	0.71	0.69	0.71
Lysine	0.96	0.92	0.89	0.92	0.89
Determined Composition (% as fed)					
Crude Protein	15.66	16.18	15.69	15.71	15.25
Crude Fat	4.00	5.64	6.86	5.92	6.40
Calcium	3.47	3.08	3.58	3.67	4.62
Total Phosphorus	0.41	0.46	0.50	0.46	0.51
Sodium	0.11	0.13	0.14	0.15	0.13

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal

[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

[4]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided the following per g included in the diet: 2800 cellulase units, 400 mannanase units, 50 galactanase units, 1000 xylanase units, 600 glucanase units, 2500 amylase units and 200 protease units.

[5]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided 5000 phytase units per g.

Table 3.4. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets without enzyme for phase 2 (42 to 49 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	53.31	49.73	46.16	49.75	46.18
Soybean Meal	22.86	15.45	8.03	15.45	8.04
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	6.05	6.07	6.03	6.06	6.01
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	3.13	3.04	3.01	3.03	3.00
Poultry Fat	1.94	3.15	4.36	3.15	4.35
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.61	0.58	0.56	0.58	0.56
Methionine Premix[3]	0.26	0.16	0.06	0.16	0.06
Iodized Salt	0.35	0.32	0.29	0.33	0.30
Total	100.01	100.00	100.00	100.01	100.00
Calculated Composition (% as fed)					
Protein	15.70	15.70	15.70	15.70	15.70
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	4.10	4.10	4.10	4.10	4.10
Non-Phytate Phosphorus	0.37	0.37	0.37	0.37	0.37
Methionine + Cystine	0.67	0.67	0.67	0.67	0.67
Lysine	0.89	0.86	0.84	0.86	0.84
Determined Composition (% as fed)					
Crude Protein	15.24	15.26	15.84	15.70	15.37
Crude Fat	4.27	5.66	6.96	5.62	6.73
Calcium	4.97	5.30	3.97	3.81	3.97
Total Phosphorus	0.44	0.51	0.54	0.48	0.54
Sodium	0.15	0.17	0.16	0.14	0.13

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal

[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 3.5. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets with enzyme for phase 2 (42 to 49 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	53.72	50.13	46.56	50.14	46.58
Soybean Meal	22.79	15.38	7.97	15.38	7.98
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	6.26	6.22	6.17	6.21	6.15
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	3.13	3.12	3.08	3.10	3.07
Poultry Fat	1.81	3.02	4.23	3.02	4.22
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.13	0.11	0.08	0.11	0.08
Methionine Premix[3]	0.26	0.16	0.06	0.16	0.06
Superzyme OM[4]	0.05	0.05	0.05	0.05	0.05
Biophytase[5]	0.01	0.01	0.01	0.01	0.01
Iodized Salt	0.35	0.32	0.29	0.33	0.30
Total	100.01	100.02	100.00	100.01	100.00
Calculated Composition (% as fed)					
Protein	15.70	15.70	15.70	15.70	15.70
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	4.10	4.10	4.10	4.10	4.10
Non-Phytate Phosphorus	0.27	0.27	0.27	0.27	0.27
Methionine + Cystine	0.67	0.67	0.67	0.67	0.67
Lysine	0.89	0.86	0.84	0.86	0.84
Determined Composition (% as fed)					
Crude Protein	16.52	15.87	15.68	16.81	16.05
Crude Fat	4.75	5.71	6.88	5.97	6.96
Calcium	3.24	4.73	4.35	3.51	5.01
Total Phosphorus	0.37	0.39	0.44	0.42	0.44
Sodium	0.14	0.15	0.14	0.15	0.14

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal
[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.
[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.
[4]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided the following per g included in the diet: 2800 cellulase units, 400 mannanase units, 50 galactanase units, 1000 xylanase units, 600 glucanase units, 2500 amylase units and 200 protease units.
[5]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided 5000 phytase units per g.

Table 3.6. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets without enzyme for phase 3 (50 to 61 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	54.87	51.23	47.71	51.30	47.74
Soybean Meal	21.51	14.10	6.69	14.11	6.70
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	6.13	6.09	6.05	6.08	6.02
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	3.07	3.05	3.02	3.04	3.01
Poultry Fat	1.75	2.96	4.16	2.95	4.15
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.57	0.55	0.53	0.55	0.52
Methionine Premix[3]	0.25	0.15	0.05	0.15	0.05
Iodized Salt	0.35	0.32	0.29	0.33	0.30
Total	100.00	99.95	100.00	100.01	99.99
Calculated Composition (% as fed)					
Protein	15.20	15.20	15.20	15.20	15.20
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	4.10	4.10	4.10	4.10	4.10
Non-Phytate Phosphorus	0.36	0.36	0.36	0.36	0.36
Methionine + Cystine	0.65	0.65	0.65	0.65	0.65
Lysine	0.85	0.82	0.80	0.82	0.80
Determined Composition (% as fed)					
Crude Protein	17.21	16.80	16.24	15.81	15.55
Crude Fat	4.82	5.90	7.88	5.82	6.96
Calcium	2.90	3.35	4.19	3.68	5.17
Total Phosphorus	0.47	0.53	0.60	0.53	0.59
Sodium	0.14	0.16	0.20	0.13	0.15

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal
[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.
[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 3.7. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets with enzyme for phase 3 (50 to 61 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	55.26	51.68	48.11	51.69	48.13
Soybean Meal	21.45	14.04	6.62	14.04	6.63
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	6.28	6.23	6.19	6.22	6.16
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	3.14	3.12	3.09	3.11	3.08
Poultry Fat	1.62	2.83	4.04	2.83	4.03
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.10	0.07	0.05	0.07	0.05
Methionine Premix[3]	0.25	0.15	0.05	0.15	0.05
Superzyme OM[4]	0.05	0.05	0.05	0.05	0.05
Biophytase[5]	0.01	0.01	0.01	0.01	0.01
Iodized Salt	0.35	0.32	0.29	0.33	0.30
Total	100.01	100.00	100.00	100.00	99.99
Calculated Composition (% as fed)					
Protein	15.20	15.20	15.20	15.20	15.20
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	4.10	4.10	4.10	4.10	4.10
Non-Phytate Phosphorus	0.26	0.26	0.26	0.26	0.26
Methionine + Cystine	0.65	0.65	0.65	0.65	0.65
Lysine	0.85	0.82	0.80	0.82	0.80
Determined Composition (% as fed)					
Crude Protein	16.72	17.45	16.31	15.40	16.30
Crude Fat	4.79	5.77	6.95	5.86	7.19
Calcium	4.26	2.84	4.21	5.08	3.55
Total Phosphorus	0.38	0.44	0.45	0.41	0.49
Sodium	0.15	0.11	0.15	0.16	0.14

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal

[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

[4]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided the following per g included in the diet: 2800 cellulase units, 400 mannanase units, 50 galactanase units, 1000 xylanase units, 600 glucanase units, 2500 amylase units and 200 protease units.

[5]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided 5000 phytase units per g.

Table 3.8. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets without enzyme for phase 4 (62 to 78 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	55.43	51.86	48.19	51.87	48.22
Soybean Meal	20.55	13.14	5.74	13.14	5.75
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	6.53	6.49	6.44	6.47	6.42
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	3.27	3.24	3.22	3.24	3.21
Poultry Fat	1.81	3.02	4.26	3.01	4.24
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.39	0.36	0.34	0.36	0.34
Methionine Premix[3]	0.17	0.08	0.02	0.08	0.02
Iodized Salt	0.35	0.32	0.29	0.32	0.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated Composition (% as fed)					
Protein	14.80	14.80	14.80	14.80	14.80
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	4.29	4.29	4.29	4.29	4.29
Non-Phytate Phosphorus	0.31	0.31	0.31	0.31	0.31
Methionine + Cystine	0.60	0.60	0.62	0.60	0.62
Lysine	0.82	0.79	0.77	0.79	0.77
Determined Composition (% as fed)					
Crude Protein	14.12	14.57	14.58	14.79	14.61
Crude Fat	4.27	5.58	6.67	5.34	6.64
Calcium	6.13	3.97	3.97	4.19	4.18
Total Phosphorus	0.40	0.44	0.48	0.44	0.49
Sodium	0.16	0.18	0.12	0.12	0.14

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal

[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

Table 3.9. Experimental diet formulations, calculated nutrient composition and determined nutrient composition of diets with enzyme for phase 4 (62 to 78 weeks)

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Ingredient (% as fed)					
Corn	55.83	52.15	48.43	52.16	48.46
Soybean Meal	20.48	13.09	5.70	13.09	5.71
Wheat	10.00	10.00	10.00	10.00	10.00
Canola Meal	-	10.00	20.00	-	-
Juncea Meal	-	-	-	10.00	20.00
Granular Limestone	6.65	6.59	6.54	6.58	6.52
Ground Limestone	1.00	1.00	1.00	1.00	1.00
Oyster Shell	3.32	3.30	3.27	3.29	3.26
Poultry Fat	1.69	2.93	4.18	2.92	4.17
MCL4[2]	0.50	0.50	0.50	0.50	0.50
Mono-dicalcium Phosphorus	0.01	0.01	0.01	0.01	0.01
Methionine Premix[3]	0.11	0.06	0.02	0.06	0.02
Superzyme OM[4]	0.05	0.05	0.05	0.05	0.05
Biophytase[5]	0.01	0.01	0.01	0.01	0.01
Iodized Salt	0.35	0.32	0.29	0.33	0.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated Composition (% as fed)					
Protein	14.80	14.80	14.80	14.80	14.80
ME_n (kcal kg^{-1})	2850	2850	2850	2850	2850
Calcium	4.29	4.29	4.29	4.29	4.29
Non-Phytate Phosphorus	0.23	0.24	0.24	0.24	0.24
Methionine + Cystine	0.57	0.59	0.62	0.59	0.62
Lysine	0.82	0.79	0.77	0.79	0.77
Determined Composition (% as fed)					
Crude Protein	14.80	14.43	15.38	15.51	14.79
Crude Fat	4.40	5.75	6.72	5.69	6.66
Calcium	5.06	5.78	4.14	4.29	4.17
Total Phosphorus	0.34	0.38	0.45	0.38	0.44
Sodium	0.16	0.14	0.15	0.15	0.14

[1]SBM = soybean meal, CM = canola meal and JM = juncea meal

[2] Premix supplied the following per kg of diet: vitamin A (retinyl acetate), 8000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K (menadione sodium bisulphite), 2.97 mg; riboflavin, 7.6 mg; pantothenic acid (DL Ca-pantothenate), 7.2 mg; vitamin B12 (cyanocobalamin), 0.12 mg; niacin, 31 mg; folic acid, 0.66 mg; choline (choline chloride), 556 mg; biotin, 0.16 mg; pyridoxine (pyridoxine HCl), 4 mg; thiamine (thiamin mononitrite), 1.94 mg; manganese (manganous oxide), 54 mg; zinc (zinc oxide), 64 mg; copper (copper sulphate), 10 mg; selenium (sodium selenite), 0.15 mg; ethoxyquin, 50 mg; wheat middlings, 2189 mg; ground limestone (38% calcium), 1900 mg.

[3]Methionine premix is composed of 50% wheat middlings and 50% DL methionine.

[4]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided the following per g included in the diet: 2800 cellulase units, 400 mannanase units, 50 galactanase units, 1000 xylanase units, 600 glucanase units, 2500 amylase units and 200 protease units.

[5]Supplied by Canadian Bio-Systems Inc., Calgary, Alberta. This provided 5000 phytase units per g.

Feed samples were taken from each phase and analyzed for crude protein, crude fat, calcium, phosphorus, and sodium content (Tables 3.2 to 3.9) at the Nova Scotia Department of Agriculture Feed Analysis Lab in Truro, Nova Scotia. Samples of canola meal and juncea meal were analysed for nutrient composition (Appendix A) by the Department of Animal Science at the University of Manitoba (Winnipeg, MN). Samples of canola and juncea were analysed for glucosinolate content (Table 3.10) by POS Bio-Sciences (Saskatoon, SK) using the method of the Canadian Grain Commission grain research laboratory (Duan and McGregor 1981). This method used gas chromatography to measure the trimethylsilyl derivatives of hydrolyzed glucosinolates.

Table 3.10. Glucosinolate content (µmol/g on an air-dry, oil-free basis) of canola meal and juncea meal

Glucosinolates	Canola Meal	Juncea Meal
Aliphatics		
3-butenyl	1.92	10.72
4-pentenyl	0.18	0.48
2-OH-3-butenyl	4.19	0.49
2-OH-4-pentenyl	0.10	-
Indolyl		
Allyl	-	0.36
CH_3-thiobutenyl	0.13	-
Phenylethyl	0.12	0.22
CH_3-thiopentenyl	0.06	-
3-CH_3-indolyl	0.27	-
4-OH-3-CH_3-indolyl	1.12	0.24
Total Glucosinolates	8.09	12.51

3.5.3 Sample Collection and Analysis

Feed consumption (FC), body weight, and egg production were determined for each 28-day period. Feed consumption was calculated by weighing feed into the feeders and weighing the feeders back at the end of each period. Body weights were measured by placing hens on the scale two at a time and averaging the weight for the cage. Hen-day egg production was calculated by recording the number of eggs laid daily, for each 28-day period. In addition to production measurements, four eggs were collected from each cage during the last day of each 28-day period for measurement of egg quality parameters

which included individual egg weights. From this data, the feed conversion ratio was calculated for each cage using the following equation:

$$Feed\ Conversion = \frac{total\ feed\ consumed}{average\ egg\ weight\ x\ total\ \#\ of\ eggs}$$

At the end of each 48-week feeding trial, two hens per cage were euthanized by cervical dislocation. Abdominal fat pads were removed and weighed.

3.5.4 Statistical Analysis

The production performance data and fat pad weights were subjected to the Proc Mixed procedure of the Statistical Analysis Systems, Inc. (Littell et al. 1996) using software version 9.3 (SAS Institute, Inc., Cary, NC, USA) with dietary treatment and supplemental enzyme as the main effects.

The following model was employed for statistical analysis of fat pad weights:

$$Y_{ij} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \epsilon_{ijk}$$

Where Y_{ij} was the variable of interest; μ was the overall mean; τ_i was the effect of the ith protein source (i = 1 – 5); γ_j was the effect of the jth dietary inclusion level of enzyme (j = 1 – 2); $\tau\gamma_{ij}$ was the effect of the interaction between protein source and dietary inclusion level of enzyme; and ϵ_{ijk} was the random effect of error with k representing replicate cages (k = 1 – 6).

For repeated measures analysis of the production data, the repeated statement of the Proc Mixed procedure was used, adding the factor of time, δ_k (with production period as the measure of time, k = 1 – 12) and all resulting interaction levels with the main effect to the above model. For the random effect of error, *l* represented replicate cages. The following model was employed for repeated measures analysis:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + \delta_k + \tau\gamma_{ij} + \tau\delta_{ik} + \gamma\delta_{jk} + \tau\gamma\delta_{ijk} + +\epsilon_{ijkl}$$

If significant main effects or interactions are found the Tukey- Kramer procedure was used to compare differences among the least-square means. The standard error of each mean (SEM) was reported with the mean. The α-level of significance was $P \leq 0.05$.

Significant two-way interactions involving period were also subjected to the slice option of SAS, which allows for comparison of meal or enzyme means within a specific period.

3.6 Results

Only significant interactions or main effects were reported in the results section. Non-significant three-way interaction tables can be found in Appendix B for WSLH and Appendix C for BSLH. Appendices B and C contain hen-housed egg production ANOVA *P*-values and letter groupings for the main effect of period.

3.6.1 Experimental Diet Analysis

All diets were analyzed in duplicate for crude protein, crude fat and mineral content (Tables 3.2 – 3.9). Analyzed diets were similar to the calculated values for crude protein, crude fat. Calcium analyzed values tended to range above and below the calculated values. Calculated values of phosphorus ranged from 0.23 to 0.37 % while analyzed values ranged from 0.34 to 0.60 %, depending on the phase of production.

Canola and juncea meals were analyzed for glucosinolate content (Table 3.10). JM was found to have more gluconapin (3-butenyl) and a greater total glucosinolate content than CM.

3.6.2 Production Performance of White-Shell Egg Laying Hens

There was a significant effect of period on feed consumption and hen-day egg production for white-shell egg laying hens (Table 3.11). Feed consumption increased throughout the trial 3 g/hen/day, while hen-day egg production decreased by 13.1 % (Table 3.12). Hen-housed egg production decreased ($P < 0.0001$) from 96.5 to 76.1 % from periods 1 to 12 throughout the trial (Appendix B). This corresponded to a decrease in number of eggs laid per cage from 163 in period 1 to 128 in period 12.

Table 3.11. ANOVA *P*-values for production performance measurements of white-shell egg laying hens				
	Feed Consumption	Body Weight	Hen-Day Egg Production	Feed Conversion
Source of Variation				
Enzyme	0.4508	0.7934	0.6918	0.6690
Meal	0.3543	0.0054	0.0533	0.9711
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Enzyme*Meal	0.8717	0.1540	0.1957	0.9367
Enzyme*Period	0.1524	0.0030	0.1323	0.0380
Meal*Period	0.0696	0.0015	0.2113	0.8099
Enzyme*Meal*Period	0.4542	0.3968	0.1037	0.8266

There was a significant enzyme x period interaction effect on body weight (Table 3.11). Since the effect was over time, the data was sliced by period to determine how enzyme affected each time period. When sliced, no significant differences could be detected using the Tukey-Kramer option.

There was a meal x period effect on body weight ($P = 0.0015$, Table 3.11) where birds fed 20 % JM had a lower body weight than birds fed SBM in periods 2 and 4 through 12 (Table 3.13). In period 10 the hens that consumed the 20 % JM diet had a lower body weight than the 20 % CM group, as well as the SBM control (Table 3.13).

Table 3.12. Means for main effect of period on production performance measurements of white-shell egg laying hens		
Period	Feed Consumption (g/hen/day)	Hen-Day Egg Production (%)
1	$111^{b} \pm 0.6$	$97.0^{a} \pm 0.32$
2	$111^{b} \pm 0.6$	$96.7^{ab} \pm 0.35$
3	$110^{b} \pm 0.7$	$95.0^{bc} \pm 0.65$
4	$110^{b} \pm 0.7$	$96.2^{ab} \pm 0.42$
5	$111^{b} \pm 0.8$	$95.5^{b} \pm 0.31$
6	$111^{b} \pm 0.9$	$92.3^{de} \pm 0.52$
7	$110^{b} \pm 1.0$	$93.8^{cd} \pm 0.56$
8	$110^{b} \pm 1.3$	$93.5^{cd} \pm 0.54$
9	$112^{ab} \pm 1.2$	$91.6^{e} \pm 0.56$
10	$111^{b} \pm 1.4$	$87.5^{f} \pm 0.66$
11	$114^{a} \pm 1.6$	$89.0^{f} \pm 0.77$
12	$114^{a} \pm 1.4$	$83.9^{g} \pm 0.74$

a-g period means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Table 3.13. Means for meal x period interaction on body weight (g) of white-shell egg laying hens

	Meal					
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period Means
Period						
0	1631 ± 16.7	1601 ± 16.7	1635 ± 16.7	1603 ± 16.7	1588 ± 16.7	1611 ± 7.5
1	1663 ± 16.8	1626 ± 16.8	1673 ± 16.8	1646 ± 16.8	1621 ± 16.8	1646 ± 7.5
2	1727^{a} ± 19.7	1661^{ab} ± 19.7	1703^{ab} ± 19.7	1663^{ab} ± 19.7	1638^{b} ± 19.7	1678 ± 8.5
3	1721 ± 20.3	1676 ± 20.3	1725 ± 20.3	1692 ± 20.3	1645 ± 20.3	1692 ± 9.1
4	1770^{a} ± 24.3	1703^{ab} ± 24.3	1732^{ab} ± 24.3	1718^{ab} ± 24.3	1657^{b} ± 24.3	1716 ± 10.2
5	1817^{a} ± 24.4	1747^{ab} ± 24.4	1749^{ab} ± 24.4	1739^{ab} ± 24.4	1666^{b} ± 24.4	1744 ± 10.0
6	1847^{a} ± 27.0	1760^{ab} ± 27.0	1768^{ab} ± 27.0	1756^{ab} ± 27.0	1692^{b} ± 27.0	1764 ± 10.9
7	1868^{a} ± 29.0	1771^{ab} ± 29.0	1768^{ab} ± 29.0	1751^{ab} ± 30.3	1691^{b} ± 29.0	1774 ± 13.5
8	1870^{a} ± 26.9	1774^{ab} ± 26.9	1789^{ab} ± 26.9	1789^{ab} ± 26.9	1718^{b} ± 26.9	1788 ± 11.5
9	1893^{a} ± 30.6	1792^{ab} ± 30.6	1796^{ab} ± 30.6	1799^{ab} ± 30.6	1738^{b} ± 30.6	1804 ± 13.8
10	1848^{a} ± 29.0	1759^{ab} ± 29.0	1807^{a} ± 29.04	1763^{ab} ± 29.0	1691^{b} ± 29.0	1773 ± 13.3
11	1822^{a} ± 28.0	1746^{ab} ± 28.0	1759^{ab} ± 28.0	1742^{ab} ± 28.0	1674^{b} ± 28.0	1748 ± 12.5
12	1820^{a} ± 26.9	1757^{ab} ± 26.9	1763^{ab} ± 26.9	1726^{ab} ± 26.9	1689^{b} ± 26.9	1751 ± 11.9
Meal means	1792 ± 21.6	1721 ± 21.6	1744 ± 21.6	1724 ± 21.6	1670 ± 21.6	

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal

a-b period x meal interaction means ± SEM within period with different superscripts are significantly different $\alpha \leq 0.05$

There was a marginal effect of meal on hen-day egg production (Table 3.11) of WSLH where the hens fed 10 % CM produced 2.8 % more eggs than the hens fed 10 % JM (Table 3.14).

Table 3.14. Means for main effect of meal on hen-day egg production (%) of white-shell egg laying hens

Meal	Hen-Day Egg Production (%)
Soybean Meal	$93.1^{ab} \pm 0.72$
10 % Canola Meal	$93.5^{a} \pm 0.72$
20 % Canola Meal	$93.3^{ab} \pm 0.72$
10 % Juncea Meal	$90.7^{b} \pm 0.72$
20 % Juncea Meal	$92.7^{ab} \pm 0.73$

a-b means ± SEM with different superscripts are significantly different α ≤ 0.05

There was an enzyme x period effect on feed conversion ratio of WHLS (*P* = 0.0380, Table 3.11). In period 6, hens that consumed enzyme were more efficient (had a lower FCR) than hens that did not consume supplemental enzyme (Table 3.15).

Table 3.15. Means for enzyme x period interaction on feed conversion ratio (kg feed/kg egg mass) of white-shell egg laying hens

	Enzyme		
	No	Yes	Period Means
Period			
1	1.91 ± 0.029[1]	1.97 ± 0.029	$1.94^{c} \pm 0.021$
2	1.91 ± 0.029	1.94 ± 0.030	$1.92^{c} \pm 0.021$
3	1.96 ± 0.029	1.97 ± 0.029	$1.97^{bc} \pm 0.021$
4	1.90 ± 0.029	1.93 ± 0.029	$1.91^{c} \pm 0.021$
5	1.93 ± 0.029	1.91 ± 0.029	$1.92^{c} \pm 0.021$
6	**$2.01^{a} \pm 0.030$**	**$1.93^{b} \pm 0.029$**	$1.97^{bc} \pm 0.021$
7	1.94 ± 0.029	1.91 ± 0.030	$1.92^{c} \pm 0.021$
8	1.93 ± 0.029	1.90 ± 0.030	$1.92^{c} \pm 0.021$
9	1.99 ± 0.029	1.95 ± 0.029	$1.97^{bc} \pm 0.021$
10	2.03 ± 0.029	2.05 ± 0.029	$2.04^{b} \pm 0.021$
11	2.05 ± 0.030	2.03 ± 0.029	$2.04^{b} \pm 0.021$
12	2.19 ± 0.030	2.11 ± 0.030	$2.15^{a} \pm 0.021$
Enzyme Means	1.98 ± 0.020	1.97 ± 0.020	

a-c means ± SEM with different superscripts are significantly different α ≤ 0.05
[1]enzyme x period interactions are sliced for period

Meal and enzyme both had a significant effect on fat pad weight (as a percentage of body weight, Table 3.16). Enzyme increased fat pad weight by 0.55 % of the body weight (Table 3.16).

Table 3.16. AVOVA *P*-values and measurements for fat pad weight (as % body weight) of white-shell egg laying hens

	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	3.61 ± 0.371	4.89 ± 0.371	**4.25 [a] ± 0.263**
10 % Canola Meal	4.32 ± 0.371	3.75 ± 0.371	**4.04[ab] ± 0.263**
20 % Canola Meal	3.19 ± 0.371	3.50 ± 0.371	**3.35[ab] ± 0.263**
10 % Juncea Meal	3.14 ± 0.371	4.13 ± 0.371	**3.63[ab] ± 0.263**
20 % Juncea Meal	2.82 ± 0.371	3.58 ± 0.371	**3.20 [b] ± 0.263**
Enzyme Means	**3.42[b] ± 0.166**	**3.97[a] ± 0.166**	
Source of Variation			
Enzyme		0.0224	
Meal		0.0318	
Enzyme*Meal		0.1258	

a-b means ± SEM for main effects with different superscripts are significantly different $\alpha \leq 0.05$

Hens that consumed the SBM control diet had a larger fat pad (as a percentage of body weight) than hens that consumed the 20 % JM diet (Table 3.16).

No significant differences could be found for mortality data (Table 3.17). For a complete list of mortalities, including cage number, treatment number and cause, see Appendix D.

Table 3.17. ANOVA *P*-values for causes of mortality of white-shell egg laying hens

	Cause of Mortality					
	Total	FLHS[1]	Prolapse	Rickets	Septicemia	Other
Source of Variation						
Enzyme	0.8490	0.9999	0.2623	0.6431	0.3221	0.7403
Meal	0.9998	0.2112	0.5861	0.7045	0.4164	0.5809
Period	0.9836	0.8747	0.6741	0.3877	0.4449	0.3533
Enzyme*Meal	0.3797	0.7745	0.6846	0.1344	0.4164	0.8156
Enzyme*Period	0.8811	0.3715	0.4689	0.8021	0.4449	0.9236
Meal*Period	0.1355	0.5545	0.4290	0.6035	0.4749	0.7039
Enzyme*Meal*Period	0.5252	0.3936	0.4067	0.4085	0.4749	0.1500

[1]FLHS = fatty liver hemorrhagic syndrome

3.6.3 Production Performance of Brown-Shell Egg Laying Hens

There was an enzyme x period interaction effect (P = 0.0011, Table 3.18) on feed consumption of brown-shell egg laying hens. In periods 2, 4, 6 and 7, hens that consumed supplemental enzyme had lower feed consumption then hens which were not fed the enzyme (Table 3.19).

Table 3.18. ANOVA *P*-values for production performance measurements of brown-shell egg laying hens

	Feed Consumption	Body Weight	Hen-Day Egg Production	Feed Conversion
Source of Variation				
Enzyme	0.2803	0.0440	0.6056	0.4628
Meal	0.4151	0.2887	0.5732	0.8930
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Enzyme*Meal	0.4010	0.4947	0.4617	0.2951
Enzyme*Period	0.0011	0.0923	0.8524	0.0366
Meal*Period	0.0086	0.0328	0.3585	0.8496
Enzyme*Meal*Period	0.1769	0.8827	0.4312	0.2786

There was a significant meal x period interaction effect on feed consumption (Table 3.18) where hens which were fed the 20 % JM treatment consumed less feed than the SBM control or 10 % CM fed hens (Table 3.20).

Enzyme has an effect on body weight (P = 0.0440, Table 3.18) where body weight was reduced from 2146 g to 2089 g when enzymes were included in the diet. The standard error of the means for these values was 19.3 g.

There was a meal x period interaction effect on body weight (P = 0.0328, Table 3.18). However, when the data was sliced, no significant interactions could be found. The means for body weight averaged 2117 g (Table 3.21).

Period had a significant effect on hen-day egg production (Table 3.18). Hen-day egg production decreased by 15.1 % from period 1 to the end of the trial (period 12, Table 3.22). Hen-housed egg production decreased from 96.5 to 76.3 % throughout the trial (Appendix C). This corresponded to a decrease in number of eggs laid per cage from 135 in period 1 to 106 in period 12.

Table 3.19. Enzyme x period means for feed consumption (g/hen/day) of brown-shell egg laying hens

	Enzyme		
	No	Yes	Period Means
Period			
1	115 ± 1.0	114 ± 1.0	115 ± 0.7
2	**116 [a] ± 0.9**	**114 [b] ± 0.9**	115 ± 0.6
3	112 ± 1.2	110 ± 1.2	111 ± 0.8
4	**114 [a] ± 1.1**	**110 [b] ± 1.1**	112 ± 0.8
5	111 ± 1.0	108 ± 1.0	109 ± 0.7
6	**113 [a] ± 1.2**	**109 [b] ± 1.2**	111 ± 0.8
7	**111 [a] ± 1.0**	**107 [b] ± 1.0**	109 ± 0.7
8	112 ± 1.1	110 ± 1.1	111 ± 0.8
9	110 ± 1.2	110 ± 1.2	110 ± 0.9
10	108 ± 1.2	109 ± 1.2	108 ± 0.9
11	110 ± 1.3	113 ± 1.3	111 ± 0.9
12	112 ± 1.5	114 ± 1.5	113 ± 1.1
Enzyme Means	112 ± 0.9	111 ± 0.9	

a-b means ± SEM within enzyme x period slices with different superscripts are significantly different $\alpha \leq 0.05$

Table 3.20. Means for meal x period interaction effect on feed consumption (g/hen/day) of brown-shell egg laying hens

	Meal				
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM
Period					
1	116 ± 1.6	114 ± 1.6	115 ± 1.6	115 ± 1.6	112 ± 1.6
2	116 ± 1.4	116 ± 1.4	115 ± 1.4	116 ± 1.4	111 ± 1.4
3	**113 [a] ± 1.8**	**115 [a] ± 1.8**	**110 [ab] ± 1.8**	**112 [ab] ± 1.8**	**106 [b] ± 1.8**
4	113 ± 1.7	112 ± 1.7	112 ± 1.7	114 ± 1.7	109 ± 1.7
5	111 ± 1.5	108 ± 1.5	110 ± 1.5	111 ± 1.5	107 ± 1.5
6	112 ± 1.8	111 ± 1.8	112 ± 1.8	110 ± 1.8	109 ± 1.8
7	109 ± 1.6	108 ± 1.6	110 ± 1.6	111 ± 1.6	107 ± 1.6
8	111 ± 1.8	109 ± 1.8	113 ± 1.8	111 ± 1.8	111 ± 1.8
9	109 ± 1.9	109 ± 1.9	113 ± 1.9	111 ± 1.9	109 ± 1.9
10	110 ± 1.9	107 ± 1.9	113 ± 1.9	106 ± 1.9	106 ± 1.9
11	111 ± 2.1	109 ± 2.1	115 ± 2.1	111 ± 2.1	111 ± 2.1
12	114 ± 2.4	111 ± 2.4	117 ± 2.5	113 ± 2.4	111 ± 2.4
Meal Means	112 ± 1.4	111 ± 1.4	113 ± 1.4	112 ± 1.4	109 ± 1.4

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal

a-b means ± SEM within meal x period slices with different superscripts are significantly different $\alpha \leq 0.05$

Table 3.21. Means for meal x period interaction effect on body weight (g) of brown-shell egg laying hens

	Meal					
	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period Means
Period						
0	1966 ± 25.8	2002 ± 25.8	1970 ± 25.8	2015 ± 25.8	2002 ± 25.8	1991 ± 11.5
1	2025 ± 32.5	2063 ± 32.5	2029 ± 32.5	2099 ± 32.5	2064 ± 32.5	2056 ± 14.5
2	2079 ± 27.3	2127 ± 27.3	2066 ± 27.3	2105 ± 27.3	2082 ± 27.3	2092 ± 12.2
3	2081 ± 26.8	2127 ± 26.8	2063 ± 26.8	2101 ± 26.8	2091 ± 26.8	2093 ± 12.0
4	2120 ± 33.0	2151 ± 33.0	2045 ± 33.0	2130 ± 33.0	2095 ± 33.0	2108 ± 14.8
5	2209 ± 42.5	2235 ± 42.2	2094 ± 42.2	2153 ± 42.2	2154 ± 42.2	2169 ± 18.9
6	2156 ± 33.0	2202 ± 33.0	2085 ± 33.0	2159 ± 33.0	2144 ± 33.0	2149 ± 14.7
7	2150 ± 38.0	2216 ± 37.8	2082 ± 37.8	2149 ± 37.8	2146 ± 37.8	2149 ± 16.9
8	2195 ± 40.3	2245 ± 40.3	2092 ± 40.3	2207 ± 40.3	2167 ± 40.3	2181 ± 18.0
9	2166 ± 39.1	2217 ± 39.1	2088 ± 39.1	2180 ± 39.1	2171 ± 39.1	2164 ± 17.5
10	2126 ± 32.9	2164 ± 32.9	2065 ± 32.9	2154 ± 32.9	2118 ± 32.9	2125 ± 14.7
11	2124 ± 32.6	2146 ± 32.6	2067 ± 32.6	2145 ± 32.6	2132 ± 32.6	2123 ± 14.6
12	2143 ± 32.7	2168 ± 32.7	2087 ± 32.7	2120 ± 32.7	2111 ± 32.7	2126 ± 14.6
Meal Means	2119 ± 30.5	2159 ± 30.5	2064 ± 30.5	2132 ± 30.5	2114 ± 30.5	

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal

Table 3.22. Means for main effect of period on hen-day egg production (%) of brown-shell egg laying hens	
Period	Hen-Day Egg Production (%)
1	$96.5^{a} \pm 0.56$
2	$95.9^{a} \pm 0.62$
3	$95.4^{a} \pm 0.51$
4	$93.5^{b} \pm 0.63$
5	$90.7^{c} \pm 0.77$
6	$88.9^{cd} \pm 0.79$
7	$88.5^{d} \pm 0.77$
8	$89.3^{cd} \pm 0.83$
9	$87.5^{de} \pm 0.90$
10	$82.9^{fg} \pm 0.93$
11	$84.9^{ef} \pm 1.07$
12	$81.4^{f} \pm 1.10$

a-f means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

There was an enzyme x period interaction on feed conversion ratio of BSLH where hens that consumed the enzymes had a lower FCR than hens that were not fed the enzymes in period 5 (Table 3.23).

Table 3.23. Means for enzyme x period interaction effect on feed conversion ratio (kg feed/kg egg mass) of brown-shell egg laying hens			
	Enzyme		
	No	Yes	Period Means
Period			
1	1.96 ± 0.030	1.97 ± 0.030	$1.96^{c} \pm 0.021$
2	1.96 ± 0.030	1.95 ± 0.030	$1.96^{cd} \pm 0.021$
3	1.92 ± 0.030	1.86 ± 0.030	$1.89^{d} \pm 0.021$
4	1.98 ± 0.030	1.93 ± 0.030	$1.95^{cd} \pm 0.021$
5	**$2.01^{a} \pm 0.030$**	**$1.91^{b} \pm 0.030$**	$1.95^{cd} \pm 0.021$
6	2.03 ± 0.030	1.98 ± 0.030	$2.00^{c} \pm 0.021$
7	1.97 ± 0.030	1.94 ± 0.030	$1.95^{cd} \pm 0.021$
8	1.99 ± 0.030	1.96 ± 0.030	$1.98^{c} \pm 0.021$
9	1.99 ± 0.030	1.97 ± 0.030	$1.98^{c} \pm 0.021$
10	2.06 ± 0.030	2.10 ± 0.030	$2.08^{b} \pm 0.021$
11	2.08 ± 0.030	2.09 ± 0.030	$2.09^{b} \pm 0.021$
12	2.20 ± 0.030	2.25 ± 0.030	$2.22^{a} \pm 0.021$
Enzyme Means	2.01 ± 0.021	1.99 ± 0.021	

a-d means ± SEM for period x enzyme slices and among meal main effects with different superscripts are significantly different $\alpha \leq 0.05$

Since this interaction was only significant in one period, the overall effect of period was also analysed. Generally, brown-shell egg laying hens had a poorer (higher value) FCR at the end of the trial compared to the beginning (Table 3.23).

There were no significant differences found for fat pad weight as a percentage of body weight. Means averaged 3.69 % (Table 3.24).

Table 3.24. AVOVA *P*-values and measurements for fat pad weight (as % body weight) of brown-shell egg laying hens

	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	4.41 ± 0.375	3.99 ± 0.375	4.20 ± 0.265
10 % Canola Meal	4.37 ± 0.347	3.94 ± 0.375	3.16 ± 0.255
20 % Canola Meal	4.14 ± 0.410	3.24 ± 0.375	3.69 ± 0.278
10 % Juncea Meal	4.16 ± 0.375	3.83 ± 0.375	4.00 ± 0.265
20 % Juncea Meal	3.67 ± 0.375	3.82 ± 0.375	3.75 ± 0.265
Enzyme Means	4.20 ± 0.168	4.15 ± 0.168	
Source of Variation			
Enzyme		0.1125	
Meal		0.5550	
Enzyme*Meal		0.7567	

There were no significant treatment effects on cause of mortality for brown-shell egg laying hens (Table 3.25). Similar to WHLS, common causes of mortality included fatty liver hemorrhagic syndrome, prolapse, rickets, septicemia from *E. coli* or *Staphylococcus aureus* or other. For a complete list of mortalities, see Appendix D.

Table 3.25. ANOVA *P*-values for causes of mortality of brown-shell egg laying hens

	Cause of Mortality					
	Total	FLHS[1]	Prolapse	Rickets	Septicemia	Other
Source of Variation						
Enzyme	0.7207	0.3235	0.9999	0.3235	0.5674	0.2273
Meal	0.3928	0.8998	0.5716	0.4546	0.2688	0.4854
Period	0.8157	0.2409	0.5363	0.4501	0.6266	0.7129
Enzyme*Meal	0.1053	0.4444	0.3258	0.4546	0.8701	0.0861
Enzyme*Period	0.6848	0.9317	0.3712	0.4501	0.3966	0.5115
Meal*Period	0.0937	0.6161	0.5021	0.6043	0.5790	0.7040
Enzyme*Meal*Period	0.8486	0.2957	0.5780	0.6043	0.4109	0.7925

[1]FLHS = fatty liver hemorrhagic syndrome

3.7 Discussion

3.7.1 Experimental Diet Analysis

When diets were analysed for Ca content, results ranged both above and below calculated Ca values (Tables 3.2 – 3.9). This may have been due to the fact that small particle ground limestone and larger particle oyster shell were used as the sources of Ca. Diets were ground before analysis but it was possible that the oyster shell was not ground as finely as desired, resulting in larger pieces. When samples were analysed, these larger pieces may have been included. This would result in analysed Ca values which were larger than calculated Ca values. Analysed Ca values lower than the calculated Ca values could have been due to the ground limestone, which tends to settle out during analysis. This could lead to less Ca being included during analysis, resulting in lower analysed Ca values. Although analysed Ca values fluctuated, there was no indication that hens were not receiving the appropriate amount of dietary Ca.

Calculated available P values (in the form of NPP) ranged from 0.23 to 0.37 % (Tables 3.2 – 3.9), depending on which phase in the production cycle the hens were in. The NRC (1994) recommends that hens receive 0.25 % NPP when diets are formulated for a FC of 100 g/hen/day and 0.21 % when diets are formulated for a FC of 120 g/hen/day. Diets for phase 1 and 2 of the current trial were formulated based on a FC of 105 g/hen/day while diets for phases 3 and 4 were based on a FC of 110 g/hen/day. Therefore, the calculated NPP values of all diets agree with the nutrient requirements listed in the NRC for poultry (1994). The production performance, egg quality and bone health results did not indicate any problems that would be associated with a P deficiency.

3.7.2 Production Performance of White-Shell Egg Laying Hens

Over the course of the trial, feed consumption increased for WSLH to 114 g/hen/day. This was expected because laying hens tend to consume more feed as they age. This increase in feed consumption is still within the breeder recommended range of 105 – 115 g/hen/day during the production period (Lohmann Tierzucht 2010).

There was a significant meal x period interaction effect on body weight where in periods 4 and 6-12, hens that consumed the 20 % JM treatment had a reduced body weight compared to hens that consumed the SBM control. Similarly, Cheva-Isarakul et

al. (2001) found that including 20 % mustard meal in diets of laying hens caused a reduction in body weight of 26.7 g compared to the control hens. In the study by Cheva-Isarakul et al. (2001) the decrease in hen weight was accompanied by a reduction in feed intake. There was no effect of meal (either as an interaction or a main effect) on feed consumption for WSLH, indicating that the reduction in body weight in the current study was not due to a decrease in feed intake.

Although there was a reduction in body weight, the weight of the 20 % JM fed hens for each period was above the body weight suggested by the breeder for the same age (Table 3.26). Since the reduction in body weight was not accompanied by a reduction in egg production or feed conversion, it did not appear to be harmful to the laying hen.

Table 3.26. Body weights of hens (g) fed each meal for periods 0 through 12 and the respective breeding company body weight (g) guidelines

	Meal					Lohmann
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Guidelines[2]
0	1631	1601	1635	1603	1588	1571
1	1663	1626	1673	1646	1621	1581
2	1727	1661	1703	1663	1638	1589
3	1721	1676	1725	1692	1645	1597
4	1770	1703	1732	1718	1657	1601
5	1817	1747	1749	1739	1666	1605
6	1847	1760	1768	1756	1692	1609
7	1868	1771	1768	1751	1691	1613
8	1870	1774	1789	1789	1718	1616
9	1893	1792	1796	1799	1738	1618
10	1848	1759	1807	1763	1691	1620
11	1822	1746	1759	1742	1674	1622
12	1820	1757	1763	1726	1689	1624

[1] SBM = soybean meal, CM = canola meal, JM = juncea meal
[2] Lohmann Tierzucht (2007)

There was a significant enzyme x period interaction on body weight. However, when the Tukey-Kramer test was performed to compare differences among the least squares means, the interaction could not be detected. This may have been because interactions can be affected by the amount of data analyzed. In this analysis there were 2 enzyme combinations within each period. Tukey-Kramer controls the type I error for the entire experiment (experimental error) as opposed to a less conservative test like LSD,

which controls type one error for the comparison only. With LSD, when you set an α-value (ie. *P* = 0.05), this is the value tested. However, with the Tukey-Kramer method each comparison made slightly reduced the α-value tested. As the number of pairs increased, the probability of seeing differences due to treatment decreased, which may explain why ANOVA recognized treatment differences that were not detected at either the 0.1 or 0.05 level during comparison of least squares means.

There was a marginal effect of meal on egg production where hens fed 10 % JM produced fewer eggs than hens fed 10 % CM. However, the percent egg production for 10 % CM and 10 % JM was not different from the SBM control. Marangos et al. (1974) found a similar result when they compared 12 % *Brassica napus* meal to 12 % *Brassica juncea* meal. The *B. juncea* meal fed hens produced fewer eggs than the *B. napus* fed hens but neither group was different from the corn-SBM control or the commercial laying hen diets being used at the time.

There was a significant effect of period on egg production, where hen-day production decreased throughout the trial. In period 12 (78 weeks of age) hen day egg production was at 83.9 %. Cheva-Isarakul et al. (2001) reported hen-day egg production values of 80.0 to 86.7 % when hens were 50 to 62 weeks of age. Similarly, Jia et al. (2008) reported egg production values of 81.9 to 82.7 % when hens were 63 weeks old. This indicates that hens in the current trial were able to maintain a higher rate of production than was typical in other flocks.

There was a significant enzyme x period interaction effect on feed conversion where in period 6, enzyme supplementation improved FCR. Han et al. (2010) found that including a multicarbohydrase enzyme in a moderate energy diet improved the FCR compared to the moderate energy diet without enzyme. Jia et al. (2008) included a multicarbohydrase but did not report an effect on FCR of laying hens. Jalal and Scheideler (2001) found that including phytase in the improved the FCR of laying hens. However, the control diets used in the study by Jalal and Scheideler (2001) were deficient in available P, which could explain the improvement of FCR when phytase was fed. The control diets fed in the current study were not deficient in P but an improvement in FCR was still reported. Other studies reported no effect of phytase on FCR of laying hens

when diets were not deficient in NPP (van der Klis et al. 1997, Um and Paik 1999 and Silversides and Hruby 2009).

Since the enzyme x period interaction was only significant in one period, the main effect of period was also reported. Over the trial FCR increased, meaning hens became less efficient. This was expected as the hens aged. However, Lohmann Tierzucht (2010) suggests that hens should maintain a FCR of less than 2.1 throughout the laying period. Only in period 12 (at 78 weeks of age) did the WSLH have a FCR above this threshold.

Meal had a significant effect on the weight of the abdominal fat pad (as a percentage of body weight) where 20 % JM had a lower ratio than the SBM control. This followed the same general trend as body weight of WSLH (Table 3.13). Cheva-Isarakul et al. (2001) found that including 20 % mustard meal in the diet reduced body weight but increased the weight of the abdominal fat. However, a study done with broiler breeder hens found that *ad lib*. fed hens had a greater body weight and fat pad weight then hens that were fed exactly to the broiler-breeder guidelines (Sun et al. 2006). The hens in the current study were fed based on the breeder guidelines, but feed was never restricted, which could explain why the body weight of all groups was above that recommended by the breeder.

A study done with rats evaluated the effects of specific glucosinolates with the addition of myrosinase (which converts glucosinolates to their more harmful breakdown products). The study found that when gluconapin (3-butenyl) was fed there was a trend towards a reduction in body weight, but there was no effect on feed consumption (Bjerg et al. 1989). Since the digestive system of rats and laying hens are not identical, there may have been a more significant effect on the reduction in body weight for the laying hens in the current trial than was found with rats. Finally, there was a difference in glucosinolate concentration between the CM and JM used in the current study. JM had more gluconapin than CM (10.92 and 1.92 μmol/g, respectively, Table 3.10).

There were no treatment effects on mortality of WSLH. The most common causes of mortality were fatty liver hemorrhagic syndrome, prolapse, rickets, septicemia from *E. coli* or *Staphylococcus aureus* or other.

3.7.3 Production Performance of Brown-Shell Egg Laying Hens

The same performance measurements were recorded for brown- and white-shell egg laying hens. Therefore, only measurements which differed from the WSLH will be discussed in this section. For discussion on other measurements, see Section 3.7.1.

There was a significant enzyme x period interaction on feed consumption where in periods 2, 4, 6 and 7, hens fed diets containing enzyme consumed less feed than hens not fed enzyme. Jia et al. (2008) found that including Superzyme-OM™ in a diet which also contained full-fat canola seed reduced feed consumption from 94.6 g/hen/day to 91.7 g/hen/day. Several studies have reported that including phytase in the diet had no effect on feed consumption (van der Klis et al. 1997 and Silversides and Hruby 2009) while others have reported that feed consumption increased when hens were fed the enzyme (Um and Paik 1999).

The decrease in feed consumption which resulted from the consumption of enzyme was expected because the purpose of the enzymes was to reduce the nutrient binding capacity of the diets, allowing the hens to receive more nutrients from a smaller amount of feed. During production, the breeder suggests that the BSLH should be consuming between 110 and 120 g/hen/day (Lohmann Tierzucht 2010). In periods 2 and 4, the feed consumption of the hens stayed within this range, but in periods 6 and 7 they consumed 109 and 107 g/hen/day, respectively. Although the feed consumption fell outside the recommended range, there was no effect of enzyme on egg production or FRC at these periods. There was a significant effect of enzyme on hen body weight, where hens consuming the enzyme weighed 57 g less overall than hens which did not consume the enzyme. Lohmann indicated that the Lohmann Brown-Lite hens should weigh between 1900 and 2100 g at the end of production (Lohmann Tierzucht 2010). The body weight of hens which consumed the enzyme was within in this range, while the other hens were too heavy. All of the production performance data indicated that inclusion of enzyme reduced feed consumption without negatively impacting other performance traits.

There was a significant meal x period interaction effect on feed consumption where hens fed the 20 % JM diet consumed less feed than the SBM or 10 % CM fed hens in period 3. Only the 20 % JM fed hens had feed consumptions which were below the

ranges recommended by Lohmann (110-120 g/hen/day, Lohmann Tierzucht 2010). However, there was no effect of meal on body weight, egg production or FRC indicating that this decline in feed consumption did not negatively impact the production performance of the hens.

Previous studies which fed CM to laying hens did not report any change in feed consumption when CM was fed at 10 % (Summers et al. 1987) or when CM was used to completely replace SBM (Leeson et al. 1987b). Cheva-Isarakul et al. (2001) found that including 20 % mustard meal in the diet resulted in a lower feed consumption than the corn-SBM control, but that 10 % mustard meal was not different from either the control or the 20 % level. These results support the results found in the current study.

There was an enzyme x period interaction effect on FCR in period 5, where hens consuming enzyme had a lower FCR than hens that were not consuming the enzyme. This indicated that for the one period, hens consuming enzyme were more efficient than hens which were not consuming the enzyme. Similar to the results of feed consumption, a reduction in FCR was expected because the purpose of including the enzymes was to determine if they could aid the hens to extract more nutrients from less feed. However, this reduction in FCR in period 5 did not correspond with a significant reduction in feed consumption, egg production, or egg weight (Section 4.6.2). Furthermore, the FCR of the hens that did not consume the enzyme was already within the range recommended by Lohmann (Lohmann Tierzucht 2010). Therefore, this improvement of FCR for period 5 was not likely to be a commercially important change.

There was a significant meal x period interaction on body weight. However, when the Tukey-Kramer test was performed the interaction could not be detected ($P > 0.05$). This was similar to the enzyme x period interaction on body weight for the WSLH. For an explanation of this, see Section 3.7.1.

There were no treatment effects on egg production, fat pad weight as a percentage of body weight, or mortality for BSLH. The causes of mortality were similar to those of the WSLH and included fatty liver hemorrhagic syndrome, prolapse, rickets, septicemia from *E. coli* or *Staphylococcus aureus* or other.

3.8 Conclusions

For the white-shell egg laying hens, there was a marginal effect of meal on egg production where 10 % JM fed hens produced fewer eggs than 10 % CM fed hens, but both groups were not different from the SBM fed hens. There was significant effect of meal on body weight and percentage of abdominal fat where hens consuming 20 % JM has lower body weight and percentage of abdominal fat than hens consuming SBM. For brown-shell egg laying hens, 20 % JM reduced feed consumption compared to SBM, in period 3. Based on these results, the hypothesis that meal would not have a significant effect on any production performance parameters was rejected.

Enzyme significantly improved feed conversion ratio in period 6 for WSLH and period 5 for BSLH, and decreased feed consumption of BSLH in several periods. However, enzyme supplementation reduced body weight of BSLH and did not affect egg production for either strain of laying hen. Therefore the hypothesis that enzyme would improve FC, FCR and egg production, but would not impact other production performance parameters was rejected.

Since the decrease in body weight and egg production seen in the WSLH and the decrease in FC of the BSLH was not found to be detrimental to the overall health of the laying hens, it is recommended that up to 20 % CM or JM be included in diets of egg laying hens.

Enzyme supplementation reduced feed consumption and body weight and improved FCR of BSLH without negatively impacting production performance parameters. Therefore, enzyme should be included in the diet of BSLH but does not need to be included in diets for WSLH when using production performance to measure the usefulness of the enzymes.

Chapter 4. Effect of canola meal or juncea meal with or without supplemental dietary enzymes on egg quality of white- and brown-shell egg laying hens.

4.1 Abstract

Canola (*Brassica napus*) meal (CM) and juncea (*Brassica juncea*) meal (JM) were evaluated in two trials (one for white-shell laying hens (WSLH) and one for brown-shell laying hens (BSLH)). The trials, designed as 5x2 factorials in completely randomized design, compared the effects of CM, JM, or soybean meal (SBM), with and without enzyme supplementation, on egg quality characteristics. A total of 360 Lohmann LSL-Lite White (Trial 1, WSLH) and 300 Lohmann Brown-Lite (Trial 2, BSLH) laying hens, housed in 60 cages, were fed one of 10 isoenergetic and isonitrogenous diets (SBM, 10 % CM, 20 % CM, 10 % JM or 20 % JM with or without a dietary enzyme cocktail of Superzyme OM™ and Bio-Phytase™) for 48 weeks. Initially, and at the end of each 28 day period, 4 eggs per cage were collected for egg quality measurements including individual egg weight, specific gravity (SG), egg-shell breaking strength (SBS) using a TA.XTplus texture analyzer, albumen height using TSS OCD™ albumen height gauge and percent yolk, albumen and shell. For WSLH there was a marginal meal x period effect ($P = 0.0559$) on albumen height in period 2 where albumen height increased from 7.6 to 8.2 mm when 20 % CM was included in the diet. Meal x period had a significant effect on percent shell in period 1 where 10 % CM, 20 % CM and 20 % JM had a lower percent shell (9.7 %) than SBM (10.2 %). Percent yolk decreased ($P < 0.0001$) and percent albumen increased ($P < 0.0001$) when 20 % CM, 10 % JM or 20 % JM was included in the diet of WSLH. There was an enzyme x meal effect ($P = 0.289$) on albumen height of WSLH where albumen height for 10 % JM-E was greater (7.1 mm) than 10 % JM+E (6.7 mm). There were no treatment effects on egg weight, SG, or SBS for WSLH. There was an enzyme x meal effect on percent shell ($P = 0.0033$) and SG ($P < 0.0001$) for BSLH where 20 % CM-E had a larger percent shell and SG (10.1 % and 1.088, respectively) than 20 % CM+E (9.6 % and 1.084, respectively). There were no treatment effects on egg weight, percent yolk, percent albumen, SBS, or albumen height for BSLH. Results indicated that up to 20 % CM or JM could be included in laying hen diets with no negative impact to egg quality. Dietary enzymes did not improve any of the measures of egg quality, and were not required to bring the quality of the eggs from hens fed CM or JM up to the quality level of the SBM control. For this reason, enzymes are not necessary in the diets of white- or brown-shell egg laying hens for the purpose of improving egg quality.

Key Words: *Canola, Juncea, Phytase, Mulitcarbohydrase, Egg Quality, Poultry*

4.2 Introduction

There are several factors which could affect shell and internal egg quality including bird strain and age, moult, nutrition and stress (Roberts 2004). Much is known about how bird strain and age (Baker and Vadehra 1970, Roland 1979 and Silversides and Scott 2001), moult (Al-Batshan et al. 1994, Tona et al. 2002 and Ahmed et al. 2003) and stress (Dorminey et al. 1965 and Chen and Balnave 2001) affect egg quality, but nutritional strategies are always changing, leaving new or improved factors feed ingredients to be investigated.

One such factor is feeding meals made from *Brassica* species including rapeseed meal, canola meal and juncea (mustard) meal. Rapeseed meal, the precursor to canola meal, reduced egg quality factors including egg weight (March et al. 1972, Marangos and Hill 1976 and Leeson et al. 1978), albumen height (Thomas et al. 1978 and Hulan and Proudfoot 1980) and specific gravity (Hulan and Proudfoot 1980) when it was fed to laying hens. Similarly, canola meal has been known to have an effect on egg quality by reducing egg size (Summers et al. 1985 and Summers et al. 1987). Little research has been done to evaluate the effects of mustard meal on egg quality, but Cheva-Isarakul et al. (2001) found that including it in laying hen diets reduced egg weight.

There are many elements that could cause a change in egg quality including dietary levels of Ca and P (important for egg shell formation), vitamins A, C, D and E (important to internal egg quality), protein (albumen quality), and anti-nutritional factors (Roberts 2004) such as glucosinolates or phytate.

The anti-nutritional factors in CM and JM could be the cause of the decline in egg quality when these meals were fed to laying hens. Glucosinolates are hydrolysed into breakdown products which are responsible for bitterness (reduced palatability) and goitrogenic effects (Mithen 1992). Fibre (from seed hulls) can decrease the rate of passage of digesta through the intestinal tract and may cause thickening of the mucosa, making nutrients unavailable to the animal (Choct 2002). Finally, phytate binds P and forms complexes with other minerals and proteins, making them unavailable to laying hens.

Through the use of plant breeding programs (Stefansson and Kondra 1975 and Love et al. 1990) and commercially available multicarbohydrase and phytase enzymes

(Boling et al. 2000, Lázaro et al. 2003 and Han et al. 2010), it has been possible to reduce the effects of these anti-nutritional factors in diets for laying hens. However, the effect that enzymes in diets which contain CM and JM will have on egg quality factors still needs to be evaluated.

4.3 Objectives

1. To determine the effect of canola meal or juncea meal included at 10 or 20 % of the diet on egg quality characteristics of white- and brown-shell egg laying hens including: egg weight and specific gravity; percent yolk, shell and albumen; shell breaking strength, and albumen height.
2. To determine the effect of a supplemental dietary enzyme cocktail on egg quality characteristics of white- and brown-shell egg laying hens including: egg weight and specific gravity; percent yolk, shell and albumen; shell breaking strength, and albumen height.

4.4 Hypothesis

The inclusion of canola or juncea meal will not have a significant effect on egg quality characteristics of white- or brown-shell egg laying hens including: egg weight; percent yolk, albumen, and shell; egg specific gravity, shell breaking strength and albumen height. The inclusion of dietary enzyme will increase egg weight, percent shell, egg specific gravity, and shell breaking strength, but will not have a significant impact on percent yolk, percent albumen or albumen height of white- or brown-shell egg laying hens.

4.5 Materials and Methods

4.5.1 Animals, Diets and Husbandry

Hens used to measure egg quality were fed and cared for as described in Chapter 3. All birds were managed in accordance with the Dalhousie Agricultural Campus Animal Care and Use Committee guidelines which follow the Canadian Council on Animal Care Codes of Practice (2009).

4.5.2 Sample Collection and Analysis

Initially and at the end of each 28 day period, four eggs were collected from each cage for egg quality measurements which included individual egg weights, specific gravity by flotation in a graded series of saline solutions (ranging from 1.070 to 1.106 in increments of 0.004 (Hamilton 1982)), egg breaking strength (using a TA.XTplus texture analyzer (Texture Technologies Corp., Scarsdale, New York, USA)), albumen height using TSS OCD™ albumen height gauge (Technical Services and Supplies, Chessingham Park, Dunnington, York, England), yolk weight and shell weight. This data was used to calculate percent yolk and shell, and by subtraction, percent albumen. Haugh Units were also calculated using egg weight and albumen height in mm.

4.5.3 Statistical Analysis

Both trials were completely randomized designs. All measurements were subjected to ANOVA using the Proc Mixed procedure of SAS (Littell et al. 1996) with software version 9.3 (SAS Institute, Inc., Cary, NC, USA). Meal type and supplemental enzyme were tested as the main effects. The statistical model used for data at a given time point was:

$$Y_{ij} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \epsilon_{ijk}$$

Where Y_{ij} is the variable of interest; μ is the overall mean; τ_i is the effect of the ith meal (i = 1-5); γ_j is the effect of the jth dietary inclusion level of enzyme (j = 1-2); $\tau\gamma_{ij}$ is the effect of the interaction between meal and enzyme; and ϵ_{ijk} is the random effect of error with k representing replicate cages (k = 1-6).

Repeated measures analysis was used for by adding the factor of time, δ_k (with production period as the measure of time, k = 1-12) and all resulting interaction levels with the main effect to the above model. For the random effect of error, l represented replicate cages. The following model was employed for repeated measures analysis:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + \delta_k + \tau\gamma_{ij} + \tau\delta_{ik} + \gamma\delta_{jk} + \tau\gamma\delta_{ijk} + +\epsilon_{ijkl}$$

If significant main effects or interactions were found, the Tukey-Kramer procedure was used to compare differences among the least-square means. The standard error of each mean (SEM) was reported with the mean. The α-level of significance was $P \leq 0.05$.

Significant two-way interactions involving period were also subjected to the slice option of SAS, which allows for compassion of meal or enzyme means within a specific period.

4.6 Results

Only significant interactions or main effects were reported in the results section. Non-significant three-way interaction tables can be found in Appendix E for WSLH and Appendix F for BSLH.

4.6.1 Egg Quality Measurements for White-Shell Eggs

There was a significant main effect of period (Table 4.1) on egg weight and percent yolk, albumen and shell (Table 4.2). Egg weight increased by 5.5 g from the initial measurement to the measurement taken in period 12. Percent yolk was initially measured at 27.1 % of egg weight. During the course of the trial percent yolk fluctuated, reaching percent yolk values as much as 2.4 % greater than the initial measurement. However the final measurement was just 0.8 % greater than the initial measurement (Table 4.2).

Table 4.1. ANOVA *P*-values for egg weight and percent of egg components of white-shell eggs

	Egg Weight	Percent Yolk	Percent Albumen	Percent Shell
Source of Variation				
Enzyme	0.2776	0.2277	0.2419	0.9873
Meal	0.0631	< 0.0001	< 0.0001	0.7640
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Enzyme*Meal	0.6739	0.9972	0.9756	0.8881
Enzyme*Period	0.3377	0.5908	0.5261	0.3794
Meal*Period	0.0871	0.2461	0.1681	0.0232
Enzyme*Meal*Period	0.5275	0.2302	0.2368	0.6962

Period had an effect on percent albumen (Table 4.1) where percent albumen fluctuated during periods 1 to 11, and reached values 1.3 % lower than the initial measurement. However, the initial and final measurements were the same, resulting in no net change over the entire trial (Table 4.2). Opposite to percent yolk, percent shell decreased 0.8 % from the initial measurement to the final measurement in period 12 (Table 4.2).

Table 4.2. Means for main effect of period on egg weight and egg components of white-shell eggs

		Egg Component (% Egg Weight)		
Period	Egg Weight (g)	Yolk	Albumen	Shell
0	57.9^{i} ± 0.24	27.1^{e} ± 0.17	63.0^{a} ± 0.19	9.9^{ab} ± 0.04
1	59.0^{gh} ± 0.23	29.5^{a} ± 0.17	60.7^{e} ± 0.19	9.8^{abc} ± 0.04
2	59.7^{fg} ± 0.30	27.3^{e} ± 0.12	62.9^{a} ± 0.12	9.8^{bc} ± 0.04
3	58.4^{hi} ± 0.28	28.3^{cd} ± 0.10	61.8^{cd} ± 0.13	10.0^{a} ± 0.04
4	60.0^{fg} ± 0.29	28.2^{cd} ± 0.12	62.0^{bcd} ± 0.12	9.8^{abc} ± 0.04
5	60.5^{ef} ± 0.25	27.9^{d} ± 0.12	62.4^{ab} ± 0.13	9.8^{cd} ± 0.04
6	61.5^{cde} ± 0.34	28.0^{d} ± 0.14	62.4^{ab} ± 0.15	9.6^{d} ± 0.04
7	61.4^{de} ± 0.25	28.8^{b} ± 0.14	61.7^{d} ± 0.14	9.6^{de} ± 0.04
8	62.3^{bcd} ± 0.28	28.7^{bc} ± 0.14	61.9^{bcd} ± 0.14	9.4^{ef} ± 0.04
9	62.3^{bcd} ± 0.29	28.4^{bcd} ± 0.14	62.3^{abc} ± 0.14	9.4^{fg} ± 0.04
10	62.7^{abc} ± 0.30	28.8^{b} ± 0.13	62.0^{bcd} ± 0.13	9.3^{fg} ± 0.04
11	63.3^{ab} ± 0.32	28.1^{cd} ± 0.14	62.6^{a} ± 0.14	9.2^{gh} ± 0.04
12	63.6^{a} ± 0.35	27.9^{d} ± 0.12	63.0^{a} ± 0.14	9.1^{h} ± 0.04

a-h means ± SEM within parameters with different superscripts are significantly different $\alpha \leq 0.05$

Table 4.3. Means for main effect of meal on egg components of white-shell eggs

		Egg Component (% Egg Weight)		
Meal	Egg Weight (g)	Yolk	Albumen	Shell
Soybean Meal	61.6 ± 0.41	29.0^{a} ± 0.16	61.4^{c} ± 0.18	9.6 ± 0.06
10 % Canola Meal	60.9 ± 0.41	28.4^{ab} ± 0.16	62.0^{bc} ± 0.18	9.6 ± 0.06
20 % Canola Meal	61.0 ± 0.42	28.0^{bc} ± 0.16	62.4^{ab} ± 0.18	9.6 ± 0.06
10 % Juncea Meal	61.4 ± 0.41	28.2^{bc} ± 0.16	62.3^{ab} ± 0.18	9.5 ± 0.06
20 % Juncea Meal	59.9 ± 0.41	27.5^{c} ± 0.16	62.9^{a} ± 0.18	9.6 ± 0.06

a-c means ± SEM within parameters with different superscripts are significantly different $\alpha \leq 0.05$

There were significant main effects of meal on percent yolk and percent albumen (Table 4.1). Percent yolk of eggs from the 20 % CM and 10 and 20 % JM treatments were lower than the percent yolk of eggs produced on the SBM control treatment. The 20

% JM diet produced eggs with a percent yolk which was lower than the 10 % CM treatment but was not different from the 20 % CM or the 10 % JM diets (Table 4.3).

Percent albumen of eggs from the 20 % CM and 10 and 20 % JM treatments were higher than the percent albumen of eggs produced on the SBM control treatment. Similar to the trend found in percent yolk, the 20 % JM diet produced eggs with a percent albumen which was significantly different from the 10 % CM treatment but was not different from the 20 % CM or the 10 % JM diets (Table 4.3).

There was a meal x period effect ($P = 0.0232$, Table 4.1) on percent shell. The slice option showed that the difference due to meal was only significant in period 1. Within period 1, 10 and 20 % CM and 20 % JM were lower than SBM (but were not different from 10 % JM (Table 4.4).

Table 4.4. Mean for meal x period interaction effect on percent shell (% of egg weight) for white-shell eggs

	Meal				
Period	Soybean Meal	10 % Canola	20 % Canola	10 % Juncea	20 % Juncea
0	9.8[1]	9.8	9.9	9.9	10.0
1	**10.2[a]**	**9.7[b]**	**9.7[b]**	**9.8[ab]**	**9.7[b]**
2	9.9	9.8	9.8	9.6	9.8
3	10.0	10.1	9.8	9.9	10.0
4	9.7	9.9	9.9	9.7	9.9
5	9.7	9.7	9.7	9.7	9.7
6	9.7	9.6	9.5	9.6	9.6
7	9.5	9.6	9.6	9.4	9.6
8	9.4	9.3	9.4	9.3	9.5
9	9.4	9.3	9.4	9.4	9.4
10	9.2	9.4	9.3	9.2	9.4
11	9.4	9.1	9.1	9.3	9.2
12	9.1	9.1	9.2	9.0	9.1

[1]SEM = 0.10 for all means.
a-b meal x period means ± SEM within period with different superscripts are significantly different $\alpha \leq 0.05$

There was a significant main effect of period on egg specific gravity and shell breaking strength (Table 4.5). There was a net reduction in specific gravity throughout the trial, with some fluctuations above the initial value in period 2 (Table 4.6). Breaking strength decreased by 1.11 kg force over the trial. The lowest breaking strengths occurred in period 6 (2.86 kg force, Table 4.6).

There was a significant effect of period on albumen height (Table 4.5). When least squares means were compared, initial and period 1 means had the lowest values. This occurred because the eggs for these two time periods were stored longer than eggs measured at other time periods (Table 4.6). For this reason, these periods were removed from all further repeated measures analysis on albumen height (Table 4.6). There was a net decline in albumen height from period 2 to period 12 (Table 4.6).

Table 4.5. ANOVA *P*-values for shell quality and albumen height measurements for white-shell eggs

	Specific Gravity	Breaking Strength	Albumen Height	Albumen Height[1]
Source of Variation				
Enzyme	0.8117	0.3996	0.9887	0.9771
Meal	0.1209	0.4877	0.2349	0.2255
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Enzyme*Meal	0.1976	0.9037	0.0417	0.0289
Enzyme*Period	0.2828	0.2741	0.8116	0.6834
Meal*Period	0.8560	0.9038	0.0357	0.0559
Enzyme*Meal*Period	0.9943	0.6659	0.8974	0.8399

[1]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12

Table 4.6. Means for main effect of period on shell quality and albumen height measurements for white-shell eggs

Period	Specific Gravity[1]	Breaking Strength	Albumen Height	Albumen Height[1]
0	1.087^{b} ± 3.16	5.42^{a} ± 0.067	5.0^{g} ± 0.06	-
1	1.081^{ef} ± 3.48	5.28^{abc} ± 0.067	6.0^{f} ± 0.06	-
2	1.089^{a} ± 3.44	5.11^{bcd} ± 0.067	7.8^{a} ± 0.06	7.8^{a} ± 0.06
3	1.086^{bc} ± 3.70	4.09^{g} ± 0.067	6.6^{d} ± 0.06	6.6^{d} ± 0.06
4	1.086^{c} ± 2.65	5.37^{ab} ± 0.067	7.2^{b} ± 0.06	7.2^{b} ± 0.06
5	1.084^{d} ± 2.61	5.35^{ab} ± 0.067	7.2^{b} ± 0.06	7.2^{b} ± 0.06
6	1.083^{d} ± 2.94	2.86^{h} ± 0.068	7.1^{b} ± 0.06	7.1^{b} ± 0.06
7	1.079^{g} ± 3.66	5.16^{abcd} ± 0.067	6.8^{c} ± 0.06	6.8^{cd} ± 0.06
8	1.080^{efg} ± 3.75	4.87^{de} ± 0.067	6.8^{c} ± 0.06	6.8^{cd} ± 0.06
9	1.081^{e} ± 3.11	5.01^{cd} ± 0.067	6.8^{c} ± 0.06	6.8^{c} ± 0.06
10	1.080^{fg} ± 3.15	4.60^{ef} ± 0.067	6.2^{e} ± 0.06	6.2^{e} ± 0.06
11	1.079^{g} ± 03.77	4.41^{f} ± 0.067	6.3^{e} ± 0.06	6.3^{e} ± 0.06
12	1.080^{efg} ± 3.59	4.31^{fg} ± 0.068	6.3^{e} ± 0.06	6.3^{e} ± 0.06

[1]Specific gravity SEM are x 10^{-4}
[2]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12
a-g means ± SEM within parameters with different superscripts are significantly different $\alpha \leq 0.05$

There was a marginally significant meal x period effect on albumen height (Table 4.5). The slice option found differences among meal treatments for period 2, where 20 % CM had a higher albumen height than SBM and 10 % CM (Table 4.7).

Table 4.7. Means for meal x period interaction effect on albumen height (mm) of white-shell eggs

	Meal				
Period	Soybean Meal	10 % Canola	20 % Canola	10 % Juncea	20 % Juncea
0[1]	-	-	-	-	-
1	-	-	-	-	-
2[2]	**7.6**^{b}	**7.7**^{b}	**8.2**^{a}	**8.0**^{ab}	**7.7**^{ab}
3	6.6	6.4	6.7	6.6	6.8
4	7.4	6.8	7.3	7.3	7.2
5	7.2	7.0	7.4	7.4	7.1
6	7.0	7.0	7.4	7.0	7.3
7	6.7	6.7	6.8	6.9	6.8
8	6.8	6.9	6.9	6.8	6.6
9	6.8	6.6	7.0	7.0	6.7
10	6.3	6.2	6.2	6.4	6.1
11	6.5	6.2	6.2	6.4	6.1
12	6.3	6.4	6.2	6.3	6.2

[1]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12
[2]SEM for all means is 0.14
a-b means ± SEM within parameters with different superscripts are significantly different $\alpha \leq 0.05$

There was also an enzyme x meal effect (P = 0.0289, Table 4.5) where SBM-E, 10 % CM-E and 20 % JM-E had a lower albumen height than 10 % JM-E. When enzyme was included in the diet, this difference could not be detected. The albumen height of eggs from hens fed 10 % JM+E was lower than that of eggs from hens fed 10 % JM-E (Table 4.8).

Table 4.8. Means for meal x enzyme interaction effect on albumen height (mm) of white-shell eggs			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	$6.8^{bc} \pm 0.11$	$6.9^{abc} \pm 0.11$	6.8 ± 0.08
10 % Canola Meal	$6.7^{bc} \pm 0.11$	$6.8^{bc} \pm 0.11$	6.7 ± 0.08
20 % Canola Meal	$7.0^{ab} \pm 0.11$	$6.9^{abc} \pm 0.11$	6.9 ± 0.08
10 % Juncea Meal	$7.1^{a} \pm 0.11$	$6.7^{bc} \pm 0.11$	6.9 ± 0.08
20 % Juncea Meal	$6.6^{c} \pm 0.11$	$6.9^{abc} \pm 0.11$	6.8 ± 0.08
Enzyme Means	6.8 ± 0.05	6.8 ± 0.05	

a-c enzyme x meal interaction means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

4.6.2 Egg Quality Measurements for Brown-Shell Eggs

Period had an effect ($P < 0.0001$) on egg weight and percent yolk, albumen and shell (Table 4.9). Egg weight increased significantly by 5.2 g from the initial measurement to the period 12 measurement (Table 4.10).

Table 4.9. ANOVA *P*-values for egg weight and percent of egg components of brown-shell eggs				
	Egg Weight	Percent Yolk	Percent Albumen	Percent Shell
Source of Variation				
Enzyme	0.9366	0.2905	0.4002	0.9497
Meal	0.2090	0.5374	0.5103	0.3914
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Enzyme*Meal	0.9579	0.1247	0.5391	0.0033
Enzyme*Period	0.4502	0.2139	0.4729	0.2524
Meal*Period	0.1453	0.7036	0.4957	0.0761
Enzyme*Meal*Period	0.6285	0.8181	0.6088	0.7004

Percent yolk followed a trend similar to egg weight, where it increased from the initial measurement to the final measurement and fluctuated approximately 1.3 % higher or lower than the initial measurement between the initial and final measurements (Table 4.10).

Table 4.10. Means for main effect of period on egg weight and egg components of brown-shell eggs

		Egg Components (% Egg Weight)		
Period	Egg Weight (g)	Yolk	Albumen	Shell
0	58.8 ^{f} ± 0.33	26.7 ^{cde} ± 0.19	63.4 ^{bcde} ± 0.22	9.8 ^{abcd} ± 0.06
1	60.2 ^{e} ± 0.33	28.1 ^{a} ± 0.21	62.3 ^{g} ± 0.19	9.8 ^{bcd} ± 0.06
2	61.3 ^{de} ± 0.33	25.5 ^{f} ± 0.12	64.7 ^{a} ± 0.13	9.9 ^{abc} ± 0.04
3	61.5 ^{d} ± 0.33	26.5 ^{de} ± 0.16	63.6 ^{bcde} ± 0.16	9.9 ^{ab} ± 0.04
4	61.3 ^{de} ± 0.33	26.3 ^{e} ± 0.12	63.7 ^{bc} ± 0.14	10.0 ^{a} ± 0.04
5	61.9 ^{cd} ± 0.33	26.4 ^{de} ± 0.13	63.7 ^{b} ± 0.14	9.9 ^{abc} ± 0.04
6	62.7 ^{bc} ± 0.33	27.6 ^{ab} ± 0.16	62.6 ^{fg} ± 0.16	9.8 ^{bcd} ± 0.04
7	63.1 ^{ab} ± 0.33	27.7 ^{ab} ± 0.18	62.5 ^{fg} ± 0.20	9.9 ^{abc} ± 0.05
8	63.3 ^{ab} ± 0.33	27.8 ^{a} ± 0.14	62.3 ^{g} ± 0.15	9.8 ^{abcd} ± 0.06
9	64.0 ^{a} ± 0.33	26.9 ^{cd} ± 0.11	63.4 ^{bcde} ± 0.13	9.8 ^{bcd} ± 0.07
10	63.6 ^{ab} ± 0.33	27.2 ^{bc} ± 0.13	63.1 ^{cdef} ± 0.14	9.7 ^{cd} ± 0.05
11	64.0 ^{a} ± 0.33	27.4 ^{abc} ± 0.15	63.0 ^{def} ± 0.17	9.7 ^{cd} ± 0.06
12	64.0 ^{a} ± 0.33	27.5 ^{ab} ± 0.13	62.9 ^{ef} ± 0.14	9.6 ^{d} ± 0.04

a-g means ± SEM within parameters with different superscripts are significantly different $\alpha \leq 0.05$

Percent albumen fluctuated throughout the trial, with values as high as 64.7 % of egg weight in period 2 and as low as 62.3 % of egg weight in period 8. However, the initial and final measurements for percent albumen were not different from each other (Table 4.10).

The period 12 measurement for percent shell was not different from the initial measurement, but was different from measurements taken for periods 2 through 5, and period 7 (Table 4.10).

There was a significant enzyme x meal interaction on percent shell (Table 4.9), where 20 % CM+E had less shell when compared to 20 % CM-E. 20 % CM+E was not different from any other meal means (Table 4.11).

Period had an effect ($P < 0.0001$) on egg specific gravity, shell breaking strength and albumen height (Table 4.12). Specific gravity decreased by 0.004 from the initial measurement to the final measurement. During period 2, specific gravity increased significantly when compared to initial and period 1 measurements, then decreased again in period 3 (Table 4.13). Egg-shell breaking strength followed a trend similar to specific gravity, decreasing during the trial with fluctuations during the intermediate periods (Table 4.13).

Table 4.11. Means for enzyme x meal interaction effect on percent shell (% of egg weight) of brown-shell eggs

	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	$9.7^{ab} \pm 0.10$	$9.9^{ab} \pm 0.10$	9.8 ± 0.07
10 % Canola Meal	$9.9^{ab} \pm 0.09$	$9.9^{ab} \pm 0.10$	9.9 ± 0.06
20 % Canola Meal	$10.1^{a} \pm 0.10$	$9.6^{b} \pm 0.10$	9.9 ± 0.07
10 % Juncea Meal	$9.8^{ab} \pm 0.10$	$9.9^{ab} \pm 0.10$	9.9 ± 0.07
20 % Juncea Meal	$9.7^{ab} \pm 0.10$	$9.8^{ab} \pm 0.10$	9.7 ± 0.07
Enzyme Means	9.8 ± 0.04	9.8 ± 0.04	

a-b enzyme x meal interaction means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Similar to albumen height of white-shell eggs, albumen height of brown-shell eggs was lowest during periods 0 and 1. Normally albumen height decreases over time but between period 1 and 2, there was a large increase (Table 4.13). Since the albumen height values of periods 0 and 1 were not typical, and they had been stored longer than egg measured in other periods, this data was excluded from statistical analysis.

There was a significant effect of period on albumen height when periods 0 and 1 were excluded from statistical analysis (Table 4.12). There was a net decrease of 1.8 mm in albumen height over the whole trial, with some fluctuations between the first and last periods (Table 4.13).

Table 4.12. ANOVA *P*-values for shell quality and albumen height measurements for brown-shell eggs

	Specific Gravity	Breaking Strength	Albumen Height	Albumen Height[1]
Source of Variation				
Enzyme	0.1810	0.2043	0.9249	0.9485
Meal	0.0003	0.4760	0.2231	0.1991
Period	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Enzyme*Meal	< 0.0001	0.4916	0.6209	0.7033
Enzyme*Period	0.3294	0.5303	0.7332	0.6354
Meal*Period	0.5564	0.5911	0.8208	0.9728
Enzyme*Meal*Period	0.7932	0.6086	0.8355	0.7304

[1]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12

Table 4.13. Means for main effect of period on shell quality and albumen height measurements for brown-shell eggs

Period	Specific Gravity[1]	Breaking Strength	Albumen Height	Albumen Height[2]
0	1.088 ^{bc} ± 4.00	5.54 ^{ab} ± 0.076	3.5 ^{g} ± 0.08	-
1	1.082 ^{f} ± 3.39	5.42 ^{abc} ± 0.076	5.3 ^{de} ± 0.08	-
2	1.091 ^{a} ± 2.97	5.35 ^{bc} ± 0.076	7.0 ^{a} ± 0.08	7.0 ^{a} ± 0.08
3	1.088 ^{b} ± 3.19	4.42 ^{d} ± 0.077	6.9 ^{a} ± 0.08	6.9 ^{a} ± 0.08
4	1.088 ^{b} ± 3.43	5.72 ^{a} ± 0.076	5.9 ^{b} ± 0.08	5.9 ^{b} ± 0.08
5	1.087 ^{bcd} ± 3.29	5.54 ^{ab} ± 0.077	6.0 ^{b} ± 0.08	6.0 ^{b} ± 0.08
6	1.087 ^{bc} ± 2.83	3.43 ^{e} ± 0.076	5.5 ^{cd} ± 0.08	5.5 ^{c} ± 0.08
7	1.084 ^{ef} ± 3.87	5.38 ^{bc} ± 0.077	5.2 ^{de} ± 0.08	5.2 ^{d} ± 0.08
8	1.085 ^{de} ± 3.80	5.46 ^{ab} ± 0.076	5.5 ^{c} ± 0.08	5.5 ^{c} ± 0.08
9	1.086 ^{cde} ± 4.45	5.24 ^{bc} ± 0.076	5.7 ^{bc} ± 0.08	5.7 ^{bc} ± 0.08
10	1.084 ^{e} ± 3.56	5.15 ^{c} ± 0.076	4.8 ^{f} ± 0.08	4.8 ^{e} ± 0.08
11	1.084 ^{e} ± 4.10	4.73 ^{d} ± 0.078	5.0 ^{ef} ± 0.08	5.0 ^{de} ± 0.08
12	1.084 ^{e} ± 3.44	4.58 ^{d} ± 0.076	5.2 ^{e} ± 0.08	5.2 ^{d} ± 0.08

[1]SEM are x 10^{-4}
[2]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12
a-g means ± SEM within parameters with different superscripts are significantly different α ≤ 0.05

There was a meal*enzyme effect ($P < 0.0001$) on specific gravity (Table 4.12) where the specific gravity of eggs from the 20 % CM-E treatment was greater than the specific gravity of eggs from the 20 % CM+E treatment (Table 4.14). 20 % JM-E had a lower specific gravity than 10 and 20 % CM-E, but with enzyme included in the diet, 20 % CM had the lowest specific gravity.

Table 4.14. Means for enzyme x meal interaction effect on specific gravity of brown-shell eggs

	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	1.086 ^{bcd} ± 4.42[1]	1.087 ^{ab} ± 4.43	1.086 ± 3.13
10 % Canola Meal	1.087 ^{ab} ± 4.08	1.087 ^{ab} ± 4.42	1.087 ± 3.01
20 % Canola Meal	1.088 ^{a} ± 4.83	1.084 ^{d} ± 4.41	1.086 ± 3.27
10 % Juncea Meal	1.086 ^{abcd} ± 4.42	1.086 ^{abc} ± 4.41	1.086 ± 3.12
20 % Juncea Meal	1.085 ^{cd} ± 4.41	1.085 ^{bcd} ± 4.44	1.085 ± 3.13
Enzyme Means	1.086 ± 1.98	1.086 ± 1.98	

a-g enzyme x meal interaction means ± SEM with different superscripts are significantly different α ≤ 0.05
[1]All SEM are x 10^{-4}

4.7 Discussion

4.7.1 Egg Quality Measurements for White-Shell Eggs

Egg weight significantly increased over the course of the trial. This was expected, as hens tend to lay larger eggs as time in production increases (Roland 1979). The final egg weight (63.6 g) was very similar to the average egg weight which the breeder suggested the hens should be laying (62.0 g) after 14 months of lay (Lohmann Tierzucht 2010). Finally, the average egg weight for each period of lay was within the Canada Grade A large to extra-large category (Egg Regulations 2009), which is the most profitable to egg producers (Egg Farmers of Canada 2013).

Meal did not have an effect on egg weight in the current trial which did not agree with a study by Summers et al. (1987) who found a significant reduction in egg size and mass when half or all of the SBM protein was replaced with protein from CM. Similarly, Cheva-Isarakul et al. (2001) found a reduction in egg weight when 20 and 30 % mustard meal was included in the diet, compared to the SBM control. Leeson et al. (1987b) found that including 25 % CM in the diet did not change the egg size, which was supported by the results of the current trial.

Jia et al. (2008) fed diets containing 15 % whole canola seed, with and without dietary Superzyme-OM™ and found that there was no effect of enzyme on egg weight. Jalal and Scheideler (2001) included phytase at two levels in phosphorus deficient diets while Gordon and Roland (1998) included phytase in a diet with adequate level of NPP, with no effect on egg weight. These results are supported by the results of the current study.

There was a significant effect of meal on percent yolk where SBM had a greater percent yolk than 20 % CM, 10 % JM or 20 % JM but was not different from 10 % CM. Summers et al. (1987) found that replacing one-half or all of the SBM protein with CM protein did not have an effect on percent yolk. In a second experiment 10 % CM resulted in a lower percent yolk than SBM, but there was no attempt to make the two diets isocaloric (Summers et al. 1987).

There was no enzyme effect on percent yolk, which agreed with Jalal and Scheideler (2001) who included phytase from two different sources to give 250 or 300 FTU/kg of feed. Han et al. (2010) fed laying hens a multicarbohydrase at 0.10 % in a

moderate energy diet and found that percent yolk decreased compared to the moderate energy diet with no enzyme. Both the moderate energy diet and the enzyme-supplemented moderate energy diet produced percent yolk values that were less than the normal energy diet. The reduction in energy could explain the decrease in percent yolk and since the diets fed in the current study were not reduced in energy, this effect was not present.

Percent yolk increased by 0.8 % throughout the trial from 27.1 % to 27.9 %. This was expected as hens lay eggs with a greater yolk content as they age (Marion et al. 1964 and Akbar et al. 1983). Marion et al. (1964) found that hens in their second year of production laid eggs with 0.5 % more yolk than in the first year of production. The average yolk is approximately 27.5 % of a 'normal' egg (Kovacs-Nolan et al. 2005) which was only 0.40 % different from the initial and final values for the trial. This indicated that although there was a statistical difference due to meal and time, this difference would not be noticeable to the consumer, and was therefore not commercially important.

Similar to percent yolk, there was a significant effect of meal on percent albumen. However, the treatments which resulted in a reduced percent yolk when compared to SBM (20 % CM, 10 % JM or 20 % JM) had a greater percent albumen than SBM. Again, SBM was not different from 10 % CM. Regardless of treatment, percent albumen ranged from 61.4 to 62.9 %. Kovacs-Nolan et al. (2005) found that percent albumen is approximately 63 % of an egg. This value was very similar to the values obtained in the study and was only 1.6 % greater than the smallest percent albumen value, which was from the SBM control. Again, it is unlikely a consumer would notice this difference.

Percent albumen did fluctuate over time in this trial, but the initial and final values were exactly the same at 63.0 %, and reflect the values used by Kovacs-Nolan et al. (2005) to describe a typical egg.

There was a significant meal x period interaction effect on percent shell. However, when the data was sliced, the interaction was only found in period 1, where SBM had a higher percent shell (10.2 % shell) than 10 and 20 % CM, and 20 % JM (9.7 % shell). There was no difference between SBM and 10 % JM. Kovacs-Nolan et al. (2005) found that a typical egg has approximately 9.5 % shell. Since percent of shell is

used as an indirect indicator of shell strength (Roberts 2004), higher percent shell values are preferred. Although there was a decrease in percent shell with the 10 and 20 % CM, and 20 % JM diets, these values are still above the desired level of shell for a typical egg, and would therefore not result in an overall increase in cracked or broken eggs.

Since the meal x period interaction was only significant in one period, the main effect of period on percent shell was also analyzed. Percent shell decreased from the initial measurement (9.9 %) to the final measurement (9.1 %), which was expected because as hens age, they lay larger eggs but the amount of shell deposited remains relatively constant (Roland 1979).

Specific gravity decreased overall during the trial from 1.087 to 1.080. Similarly, there was a significant main effect of period on breaking strength where breaking strength decreased from the initial measurement the final measurement in period 12. The lowest breaking strengths occurred in period 6 (2.86 kg force). These values may have been due to a technical error, as they returned to more 'normal' levels in period 7 and remained there until the end of the trial. A decrease in specific gravity has been shown to occur over time (Roland 1979) and was expected. Since SG is an indirect measure of shell strength and breaking strength is a direct measure (Roberts 2004), and shell quality is known to decrease over time (Roland 1979) a decrease in both of these measurements was not unusual.

An egg shell could be considered thin with a SG of 1.070 or less, while a shell with a SG of 1.090 would be considered thick. A typical egg would have a shell with a SG of at least 1.080 (Bell and Weaver 2002). The SG found at the end of the trial was not significantly different from 1.080, which indicated that the strength of the shell at the end of production was still that of a typical egg.

There was no enzyme effect on egg specific gravity which agreed with the results of studies that included phytase (Jalal and Scheideler 2001) or a multicarbohydrase (Jia et al. 2008) in diets for laying hens.

There were no significant differences of shell breaking strength due to meal in this study, which was did not agree with the results of a study done in 1978 with Tower RSM. In the 1978 study, hen that consumed heat-treated, ground RSM has stronger egg shells than those which consumed SBM (Leeson et al. 1978). However, Leeson et al.

(1987b) completely replaced the protein from SBM with protein from CM and found no difference in shell strength.

The breeder suggests that the breaking strength of eggs from white-shell egg laying hens should be at least 40 N (Lohmann Tierzucht 2010), which is equivalent to 4.08 kg force. The shell breaking strength during this trial did not fall below this recommended level except in period 6, which further indicated results from this period was affected by a mechanical error.

Albumen height was measured initially, and for periods 1 through 12. However, eggs were stored for a longer period of time before initial measurements or period 1 albumen height measurements could be taken. Scott and Silversides (2000) found that longer periods of storage resulted in lower albumen heights. For this reason, albumen height measurements from periods 0 and 1 were not included in statistical analysis.

There was a marginally significant meal x period effect on albumen height but when the slice option was used, there were only significant results in period 2. The 20 % CM had a larger albumen height than SBM or 10 % CM. Previous research which involved feeding RSM did not find any significant differences on albumen height measured in HU (Leslie and Summers 1972, Olomu et al. 1975 and Hulan and Proudfoot 1980). Najib and Al-Khateeb (2004) found that including 10 % full-fat canola seed increased HU over the control, and that 30 % full-fat seed increased HU over both the control and 10 %.

Since the meal x period effect on albumen height was only significant in period 2, the main effect of period was also considered. Albumen height decreased over time, which was expected because as hens get older, albumen height decreases (Silversides and Scott 2001).

There was a meal x enzyme interaction on albumen height where 10 % JM-E had a greater albumen height than 10 % JM+E. This does not agree with results by Lázaro et al. (2003) who found there was no effect on albumen height (HU) when a multicarbohydrase enzyme was included in the diet. Similarly, Um and Paik (1999) and Jalal and Scheideler (2001) found no effect of phytase on HU. Silversides and Hruby (2009) found that phytase had no effect on albumen height measured in mm. These results do not support the results found in the current trial.

The Egg Regulations use HU to evaluate whether a batch of eggs meets Canada Grade A standards for internal quality. Eggs with an HU score of at least 67 can be considered Grade A, assuming they also meet the external quality factors (Egg Regulations 2009). The lowest albumen height in mm from each significant interaction or effect was converted to HU, to determine if the eggs would still be considered Grade A, even with the reduction in albumen height. For the meal x period interaction, SBM and 10 % CM had the lowest albumen height, which resulted in 96.2 and 96.6 HU, respectively. For the main effect of period, albumen height in period 12 was 6.3, which resulted in a HU score of 89.5. Finally, for the meal x enzyme interaction, 10 % JM+E had the lowest albumen height but gave a HU score of 91.7. These HU scores indicated that even with the reduction in albumen height, the egg would have been considered Canada Grade A, and could be sold as table eggs (Egg Regulations 2009).

Including enzyme in diets of white-shell egg laying hens may not be an economical option when using egg quality as the deciding factor. The reduction in albumen height was not considered commercially important, but was not the desired effect of the enzyme. As well, the enzyme did not improve any of the other measures of egg quality, and was not required to bring the quality of the eggs from CM or JM fed hens up to the level of the SBM control. For this reason, enzyme should not be included in the diet of white-shell egg laying hens for the purpose of improving egg quality.

4.7.2 Egg Quality Measurements for Brown-Shell Eggs

Since the same variables were measured in the brown-shell eggs as the white-shell eggs, only those variables with different results or with different thresholds were discussed in this section. For discussion on other variables, see section 4.7.1.

Similar to the results of the white-shell eggs, there was a period effect on egg weight of the brown-shell eggs where egg weight increased over the course of the trial. As with the white-shell eggs, this was expected because hens lay larger eggs as they get older (Roland et al. 1975 and Joyner et al. 1987). The final egg weight (64.0 g) was very similar to the average egg weight which the breeding company suggested the hens should be laying (62.5 to 63.5 g) after 14 months of lay (Lohmann Tierzucht 2010). These eggs weighed slightly more than the white-shelled eggs. Eggs from brown-shell laying hens

tend to be larger than eggs from white-shell laying hens (Scott and Silversides 2000). The average egg weight for each period of lay was within the Canada Grade A large to extra-large category (Egg Regulations 2009), which is the most profitable to egg producers (Egg Farmers of Canada 2013).

Percent yolk, albumen and shell for brown-shell eggs were very similar to those of the white-shell eggs. All three variables (27.5, 62.9 and 9.6 % respectively) were similar to measurements of a typical egg (27.5, 63.0 and 9.5 %, respectively, Kovacs-Nolan et al. 2005) at the end of the trial.

There was an enzyme x meal interaction on percent shell where 20 % CM-E had a greater percent shell than 20 % CM+E. These results agree with Han et al. (2010) who found that including a multicarbohydrase (α-1,6 galactosidase and β-1,4 mannanase) in a moderate energy diet for Lohmann Brown-Lite laying hens resulted in a reduction of percent shell when compared to the moderate energy diet without enzyme. Similarly, Jalal and Scheideler (2001) found that including phytase which provided 250 or 300 FTU/kg of feed resulted in reduced percent shell.

Although there was a reduction in percent shell with the addition of enzyme, the lower value was still above the amount of shell attributed to a typical egg (9.5 %, Kovacs-Nolan et al. 2005), and was not different from the SBM control treatment. This indicated that the reduction in percent shell would not result in more cracked or broken eggs, and would not be a commercially important decrease.

There was a significant enzyme x meal interaction on specific gravity which was similar to the enzyme x meal interaction for percent shell. The SG of 20 % CM-E (1.088) was greater than the SG of 20 % CM+E (1.084). One explanation for this could be that the supplemented phosphorus (and therefore the available P) was less in the diets which contained enzyme compared to those which did not (Tables 3.2 to 3.9). Possibly, the phytase enzyme released more P than was expected, causing a slight excess of P to be available. Um and Paik (1999) found that when the calculated available phosphorus decreased from 0.37 to 0.24 % (due to a reduction in supplemental phosphorus) the egg specific gravity decreased from 1.090 to 1.089. Vandepopuliere and Lyons (1992) found that total dietary P was inversely related to the egg SG. When 0.4 % total P was provided, hens laid eggs with the highest SG, but when 0.7 % total P was provided, hens laid eggs

with the lowest SG. Providing hens with 0.5 and 0.6 % total P, resulted in intermediate SG values. Boorman and Gunaratne (2001) found that excess P caused a reduction in SG, possibly due to the inhibitory effect that excess P has on absorption of Ca from the intestine and medullary bone. Since both % shell and SG were affected, and both measures indicate the amount of shell present in relation to the size of the egg (Roberts 2004), there is evidence that the amount of Ca available for shell formation was slightly reduced.

Since the SG value measured did not fall below the SG value of a typical egg (1.080, Bell and Weaver 2002) and was not different from the SBM control treatment, the reduction would not be considered commercially important.

Based on these results, including enzyme in diets of brown-shell egg laying hens may not be necessary when using egg quality as the deciding factor. The magnitude of the reduction in percent shell and shell SG were not considered commercially important, but were not the desired effect of the enzyme. As well, the enzyme did not improve any of the other measures of egg quality, and was not required to bring the quality of the eggs from CM or JM fed hens up to the level of the SBM control. For this reason, enzyme need not be included in the diet of brown-shell egg laying hens for the purpose of improving egg quality.

Similar to the white-shell eggs, there was a period effect on SG and shell breaking strength. While both measurements decreased with time, they did not decrease as far as the white-shell eggs. Scott and Silversides (2000) found that brown-shell eggs had a greater percent shell than white-shell eggs, indicating they would be stronger. SG remained above the desired value of 1.080 (Bell and Weaver 2002) and egg breaking strength did not fall below 3.57 kg force (35 N), which is the minimum breaking strength guaranteed by the breeder (Lohmann Tierzucht 2010).

Albumen height of brown-shell eggs was also affected by period, as were the white-shell eggs. However, the albumen height of the brown-shell eggs decreased further than the albumen height of the white-shell eggs (5.2 and 6.3 mm, respectively). Scott and Silversides (2000) found that the albumen height of brown-shell eggs was less than that of white-shell eggs (6.25 versus 7.22 mm). The albumen height of brown-shell eggs in period 12 was converted to HU to determine whether or not these eggs would still be

considered Canada Grade A. The HU score for period 12 was 82.9, which is still well above the 67 HU required to be considered Grade A (Egg Regulations 2009).

4.8 Conclusions

There was an effect of meal on percent yolk and percent albumen of white-shell eggs. Percent yolk decreased while percent albumen increased when 20 % CM, 10 % JM or 20 % JM was included in the diet of white-shell egg laying hens. There was a meal x period effect on percent shell in period 1 and albumen height in period 2. Percent shell decreased when 10 % CM, 20 % CM or 20 % JM was included in the diet. Albumen height was increased when 20 % CM was included. There was no effect of meal on egg weight, egg specific gravity or shell breaking strength for white-shell eggs. There were no effects of meal on the egg quality factors measured for brown-shell eggs. Therefore, the hypothesis that the inclusion of meals would not have a significant effect on any of the egg quality factors measured was rejected for the white-shell eggs, but confirmed for the brown-shell eggs.

Enzyme did not have an effect on any of the egg quality factors measured for white-shell eggs except for albumen height, 10 % JM-E had a higher albumen height than 10 % JM+E. However, the albumen height for 10 % JM+E was not different from the SBM control and was still within acceptable albumen height ranges. This was contrary to the original hypothesis that enzyme would increase egg weight, percent shell, egg specific gravity and shell breaking strength, but would not affect percent yolk, percent albumen, or albumen height.

There was an enzyme x meal effect on percent shell and egg specific gravity for brown-shell egg laying hens where 20 % CM-E had a higher percent shell and SG than 20 % CM+E. The percent shell and SG values for 20 % CM+E were within acceptable ranges for the variable measured, and did not differ from the SBM control. Enzyme did not have an effect on egg weight or shell breaking strength. These results are contrary to the hypothesis that dietary inclusion of enzyme would increase egg weight, percent shell, egg specific gravity and shell breaking strength. However, enzyme did not affect percent albumen, percent shell or albumen height, which confirms this part of the hypothesis.

Since all of the changes in egg quality related to meal were within normal ranges for the variables measured and would not result in more cracked, broken, or otherwise reduced quality eggs, it is recommended that up to 20 % CM or JM be included in diet of white- and brown-shell egg laying hens.

Based on these results, including enzyme in diets of white- or brown-shell egg laying hens was not necessary when using egg quality as a measure of performance. The reduction in percent shell and shell SG of brown-shell eggs, and the reduction in albumen height of white-shell eggs were not considered commercially important, but were not the desired effect of the enzyme. As well, the enzyme did not improve any of the other measures of egg quality, and was not required to bring the quality of the eggs from CM or JM fed hens up to the level of the SBM control. For this reason, enzyme should not be included in the diet of white- or brown-shell egg laying hens for the purpose of improving egg quality.

Chapter 5. Effect of canola meal or juncea meal with or without supplemental dietary enzymes on bone health of white- and brown-shell egg laying hens.

5.1 Abstract

Low-glucosinolate canola (*Brassica napus*) meal (CM) and juncea (*Brassica juncea*) meal (JM) were evaluated in laying hen diets. Two trials (one for white-shell laying hens (WSLH) and one for brown-shell laying hens (BSLH)) compared the effects of CM, JM or soybean meal (SBM), with (+E) and without (-E) enzyme supplementation, on bone characteristics. The trials were designed as 5x2 factorials in completely randomized design. Three-hundred sixty Lohmann LSL-Lite White (Trial 1, WSLH) and 300 Lohmann Brown-Lite (Trial 2, BSLH) laying hens, housed in 60 cages, were fed one of 10 isoenergetic and isonitrogenous diets (SBM, 10 % CM, 20 % CM, 10 % JM or 20 % JM with or without a dietary enzyme cocktail of Superzyme OM^{TM} and Bio-Phytase^{TM}) for 48 weeks. At the end of the trial (approximately 78 weeks of age) 2 hens per cage were euthanized. One tibia and one humerus per hen were removed and stored at 4°C until further analysis. Bones were cleaned by hand and fixed in formalin. Fixed bones were then measured for density and area with a Stratec XCT scanner and bone mineral content (BMC) was calculated. Bones were weighed and measured for length and width, and bone breaking strength (BBS) was determined. Correlations were performed between bone and egg-shell quality measurements. Enzyme x meal interactions resulted in a lower ($P \leq 0.10$) WSLH humeral total area for 20 % JM-E treatments (49.22 mm^2) when compared to 10 and 20 % CM-E treatments (55.82 mm^2). Enzyme supplementation for WSLH resulted in significantly lower tibia total cross-sectional area (40.80 to 39.39 mm^2) and humeral and tibia bone weight (4.8 to 4.5 and 11.1 to 10.4 g, respectively). In tibias of WSLH enzyme supplementation resulted in significantly lower cortical area (20.99 to 19.14 mm^2) and total and cortical BMC (27.38 to 25.75 and 22.38 to 20.71mg/mm, respectively) but greater ($P \leq 0.05$) cortical density (1048.1 to 1075.8 mg/cm^3) and trabecular area (17.11 to 19.05 mm^2), density (217.5 to 240.5 mg/cm^3) and BMC (3.79 to 4.51 mg/mm). For BSLH, neither meal type nor enzyme supplementation had a significant effect on any of the bone measurements ($P > 0.05$). Cortical density of WSLH humeri had a weak, inverse relationship (r = -0.2927) with egg-shell breaking strength (SBS) while cortical density of humeri of BSLH had a weak, positive relationship (r = 0.2999) with SBS. Total density (r = -0.3427), trabecular density (r = -0.3325), total BMC (r = -0.2956) and cortical BMC (r = -0.3012) of tibias of WSLH had inverse, weak relationships with SBS. Total BMC and BBS of tibia from BSLH were weakly, positively correlated with egg specific gravity (r = 0.2511 and 0.2529, respectively). Results indicated that up to 20 % CM or JM could be included in laying hen diets with no negative impact on bone quality. Dietary enzymes increased medullary bone reserves in tibias of WHLH.

Key Words: *Canola, Juncea, Phytase, QCT, Poultry*

5.2 Introduction

Canola and juncea meals contain phytate which is a mixed salt (consisting of potassium, magnesium and calcium) of phytic acid. It is considered the principal storage form of P in oilseeds (Pallauf and Rimbach 1997) but is an anti-nutritional factor for animals because it forms complexes with minerals (calcium, iron, zinc, manganese and magnesium) and proteins. The chemical structure of phytate allows it to chelate with cations, making them unavailable to animals (Khajali and Slominski 2012).

Chelation of minerals (especially Ca and P) can be a problem for laying hens because the diet supplies the majority of the Ca and P used in egg-shell formation. When the diet does not provide enough of these minerals, hens must mobilize bone to provide the rest of the Ca and P needed to form the shell. Usually, medullary bone is mobilized for this purpose, but osteoclasts are not specific to medullary bone and will resorb structural (cortical) bone if it is exposed. While medullary bone is replaced when the hen does not have an egg in the shell gland, cortical bone is not reformed unless the hen leaves the laying cycle. Over time this can lead to a weakening in bone structure, and ultimately, osteoporosis (Whitehead 2004).

The inclusion of a dietary phytase enzyme may help to break down phytate in the diet, which may lead to improved bone quality factors. A commercially available form of phytase is Bio-Phytase 5000G (Canadian Bio-Systems Inc. Calgary, Alberta).

Very little work has been done to assess the effects of CM on bone quality. A study using broiler chickens found that feeding a cold-pressed yellow-seeded RSM resulted in greater tibia weight and breaking strength than feeding a cold-pressed black-seeded RSM. However, the study also found that feeding two other yellow-seeded RSMs were not different from the black-seeded meal (Czerwiński et al. 2012). No research has been reported using CM or JM for laying hens with respect to bone quality measurements.

Some research was conducted to evaluate the use of phytase enzymes in diets for broiler chickens to improve bone quality. Czerwiński et al. (2012) found that including 1000 FYT/kg of diet improved tibia strength compared to birds which received the un-supplemented diet. However, van der Klis et al. (1997) supplemented laying hen diets with up to 300 FTU/kg of basal diet and found no change in tibia weight. No research has

been reported where CM or JM was fed to laying hens, with supplemental dietary phytase. This suggests more work needs to be done to determine what effect phytase supplementation will have on laying hens consuming a CM or JM diet.

5.3 Objectives

1. To determine the effect of canola meal or juncea meal included at 10 or 20 % of the diet on bone characteristics of white- and brown-shell egg laying hens including: bone weight, length and width; total cross-sectional area, density and bone mineral content; cortical cross-sectional area, density and bone mineral content; trabecular cross-sectional area, density and bone mineral content; and bone breaking strength.
2. To determine the effect of a supplemental dietary enzyme cocktail on bone characteristics of white- and brown-shell egg laying hens including: bone weight, length and width; total cross-sectional area, density and bone mineral content; cortical cross-sectional area, density and bone mineral content; trabecular cross-sectional area, density and bone mineral content; and bone breaking strength.

5.4 Hypothesis

The inclusion of canola or juncea meals will not have a significant effect on bone characteristics of white- or brown-shell egg laying hens including: bone weight, length and width; total cross-sectional area, density and bone mineral content; cortical cross-sectional area, density and bone mineral content; trabecular cross-sectional area, density and bone mineral content; and bone breaking strength. The inclusion of dietary enzyme will increase bone weight, cross-sectional area, density, and bone mineral content and bone breaking strength, but will not have a significant impact on bone length or width of white- or brown-shell egg laying hens.

5.5 Materials and Methods

5.5.1 Animals, Diets and Husbandry

Hens used to measure bone quality were fed and cared for as described in Chapter 3. All birds were managed in accordance with the Dalhousie Agricultural Campus

Animal Care and Use Committee guidelines which follow the Canadian Council on Animal Care Codes of Practice (2009).

5.5.2 Sample Collection and Analysis

At the end of each 48 week feeding trial, two hens per cage were euthanized by cervical dislocation. One tibia and one humerus per hen were collected and placed in a Whirl-pak bag. Bones were refrigerated at 4°C until tissue could be removed. Tissue was removed by hand, with scissors and a scalpel blade. Once cleaned, the humeri were placed in falcon tubes and the tibias were placed in Whirl-Pak bags. The bones were covered with 10 % phosphate buffered formalin (Sigma-Aldrich Canada Co., Oakville, ON) and left to fix for four weeks. The bones were removed from the formalin, rinsed with distilled water, and placed into a clean Whirl-Pak bag with a cotton ball soaked in distilled water to prevent the bones from drying out.

Bone density analysis was conducted on 8 bones per treatment in the Department of Agricultural, Food and Nutritional Sciences at the University of Alberta (Edmonton, Alberta, Canada) using a Stratec XCT scanner (model 922010, Norland Medical Systems Inc., Fort Atkinson, WI) with XMENU software version 5.40C, using the method by Korver et al. (2004). The bones were randomly selected so that 8 tibias and 8 humeri of white- and brown-shell egg laying hens per treatment were measured. The scanner was calibrated using a cone phantom with 4 known areas of x-ray attenuation. Bones were placed in a graduated cylinder which was clamped in place in the aperture. A longitudinal (scout) scan (Fig. 5.1) was used to set the location of the cross-sectional scan. The cross-sectional x-ray (Fig. 5.2) was set at 30 % from the proximal end of the bone being measured. A threshold value of 400 mg/cm^3 was used to separate cortical and subcortical bone from trabecular bone, and 500 mg/cm^3 to separate cortical bone from subcortical bone. The resulting picture from the 1mm cross-sectional x-ray (displayed on a computer monitor) indicated total, cortical, and trabecular bone densities and area, which were multiplied to give the mass (mg QCT) of total, cortical, and trabecular bone. Bone breaking strength was conducted on the same bones used for density measurements using a TA.XTplus texture analyzer (Texture Technologies Corp., Scarsdale, New York, USA) with software version 5.2, a 50 kg load cell, and a standard sheer plate.

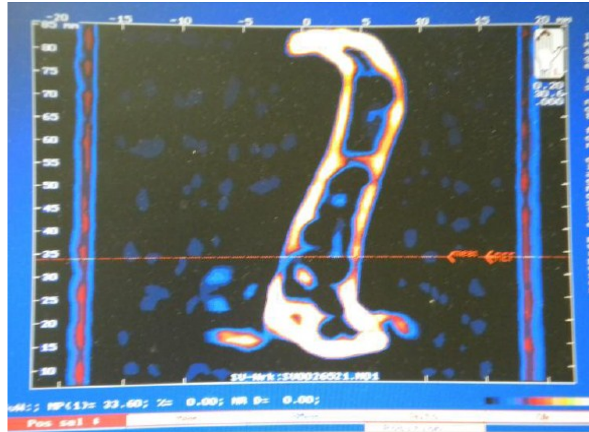

Fig. 5.1. Scout scan of humerus using a Stratec XCT scanner.

The scout (longitudinal) scan was used to set the position of the cross-sectional x-ray 30 % from the proximal end of the humerus (as indicated by the red line)

The length of the bones, and width at the midpoint were measured to the nearest mm. Bones were weighed in grams to one decimal place, and placed on the supports for bone breaking. A 2 cm distance between the two fixed points supporting the bone on the three-point bending rig was used. The force was applied to the same side of each bone and the force required to break each bone was recorded.

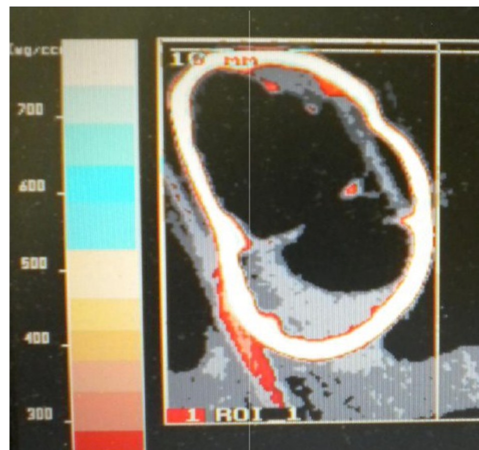

Fig. 5.2. Cross-sectional scan of humerus displayed on XMENU software.

This cross-section was taken 30 % from the proximal bone end, as indicated by the red line on Fig. 5.1.

5.5.3 Statistical Analysis

All measurements were subjected to ANOVA using the Proc Mixed procedure of the Statistical Analysis Systems (SAS), Inc. (Littell et al. 1996) with software version 9.3 (SAS Institute, Inc., Cary, NC, USA). Meal type and supplemental enzyme were tested as the main effects. The statistical model used for bone measurements was:

$$Y_{ij} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \epsilon_{ijk}$$

Where Y_{ij} is the variable of interest; μ is the overall mean; τ_i is the effect of the ith meal (i = 1-5); γ_j is the effect of the jth dietary inclusion level of enzyme (j = 1-2); $\tau\gamma_{ij}$ is the effect of the interaction between meal and enzyme; and ϵ_{ijk} is the random effect of error with k representing replicate bone measurements (k = 1-8).

If significant main effects or interactions were found, the Tukey-Kramer procedure was used to compare differences among the least-square means. The standard error of each mean (SEM) was reported with the mean. The α-level of significance was $P \leq 0.05$.

Data for tibia cortical density of brown-shell egg laying hens could not be made normal through transformations. Therefore the Kruskal-Wallis test (the non-parametric equivalent to one-way ANOVA) was used in SAS version 9.3.

Correlations were performed on bone quality measurements and egg-shell quality measurements using the Proc Corr procedure of SAS version 9.3. Cage was used as the experimental unit. Correlations were performed only on normal data so transformations were performed where necessary and outliers were removed.

5.6 Results

5.6.1 Humerus Measurements for White-Shell Egg Laying Hens

There was a significant enzyme x meal interaction for total cross-sectional area of humeri from white-shelled egg laying hens (Table 5.1). With a significance level of α = 0.05, the Tukey-Kramer procedure did not find any differences among the means. However, with a significance of α = 0.10, it was found that 10 % CM-E and 20 % CM-E,

had the same total cross-sectional area, which was greater than that for 20 % JM-E (Table 5.2).

Table 5.1. ANOVA *P*-values for humerus cross-sectional area measurements from white-shell egg laying hens

	Area		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.0475	0.5276	0.0619
Meal	0.1233	0.8373	0.1591
Enzyme*Meal	0.0452	0.0724	0.0899

There were no significant differences for cortical or trabecular area (Table 5.1) but values averaged 11.55 mm^2 and 39.51 mm^2, respectively (Table 5.2).

Analysis of variance for humerus total density measurements showed a marginally significant enzyme x meal interaction (Table 5.3), however no differences could be detected for the least squares means (Table 5.4).

The overall mean for total density was 164.1 mg/cm^3. Cortical density values were not different from one another ($P > 0.05$, Table 5.3) and averaged 987.2 mg/cm^3 (Table 5.4).

Table 5.2. Humerus cross-sectional area measurements (mm²) from white-shell egg laying hens

Total Area (mm²)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	**52.72 [ab] ± 1.497**	**51.78 [ab] ± 1. 497**	52.25 ± 1.059
10 % Canola Meal	**55.82 [a] ± 1. 497**	**49.85 [ab] ± 1. 497**	52.83 ± 1.059
20 % Canola Meal	**55.82 [a] ± 1. 497**	**50.86 [ab] ± 1. 497**	53.34 ± 1.059
10 % Juncea Meal	**50.15 [ab] ± 1. 497**	**50.73 [ab] ± 1. 497**	50.44 ± 1.059
20 % Juncea Meal	**49.22 [b] ± 1. 497**	**50.95 [ab] ± 1. 497**	50.08 ± 1.059
Enzyme Means	52.74 ± 0.670	50.84 ± 0.670	

Cortical Area (mm²)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	11.15 ± 0.398	12.12 ± 0. 398	11.63 ± 0.281
10 % Canola Meal	12.15 ± 0. 398	10.78 ± 0. 398	11.46 ± 0.281
20 % Canola Meal	11.77 ± 0. 398	11.60 ± 0. 398	11.68 ± 0.281
10 % Juncea Meal	11.85 ± 0. 398	11.52 ± 0. 398	11.68 ± 0.281
20 % Juncea Meal	11.24 ± 0. 398	11.36 ± 0. 398	11.30 ± 0.281
Enzyme Means	11.63 ± 0.178	11.47 ± 0.178	

Trabecular Area (mm²)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	40.63 ± 1.381	38.91 ± 1.381	39.77 ± 0.977
10 % Canola Meal	42.75 ± 1.381	38.34 ± 1.381	40.55 ± 0.977
20 % Canola Meal	43.11 ± 1.381	38.59 ± 1.381	40.85 ± 0.977
10 % Juncea Meal	37.57 ± 1.381	38.48 ± 1.381	38.03 ± 0.977
20 % Juncea Meal	37.62 ± 1.381	39.07 ± 1.381	38.34 ± 0.977
Enzyme Means	40.34 ± 0.618	38.68 ± 0.618	

a-b enzyme x meal interaction means ± SEM within parameters with different superscripts are significantly different 0.10 ≥ α > 0.05.

Table 5.3. ANOVA *P*-values for humerus density measurements from white-shell egg laying hens

	Density	
	Total	Cortical
Source of Variation		
Enzyme	0.4670	0.9406
Meal	0.6733	0.0924
Enzyme*Meal	0.0590	0.4059

Table 5.4. Humerus density measurements (mg/cm^3) from white-shell egg laying hens

	Total Density (mg/cm^3)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	138.8 ± 22.74	176.8 ± 22.74	156.7 ± 16.08
10 % Canola Meal	169.4 ± 22.74	135.7 ± 22.74	151.6 ± 16.08
20 % Canola Meal	143.7 ± 22.74	176.8 ± 22.74	159.4 ± 16.08
10 % Juncea Meal	201.2 ± 22.74	134.9 ± 22.74	164.8 ± 16.08
20 % Juncea Meal	192.8 ± 22.74	170.8 ± 22.74	181.4 ± 16.08
Enzyme Means	167.3 ± 10.17	157.8 ± 10.17	
	Cortical Density (mg/cm^3)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	959.4 ± 11.30	977.6 ± 11.30	968.5 ± 7.99
10 % Canola Meal	999.4 ± 11.30	979.8 ± 11.30	989.6 ± 7.99
20 % Canola Meal	989.8 ± 11.30	993.0 ± 11.30	991.4 ± 7.99
10 % Juncea Meal	1005.7 ± 11.30	993.8 ± 11.30	999.7 ± 7.99
20 % Juncea Meal	980.1 ± 11.30	993.0 ± 11.30	986.5 ± 7.99
Enzyme Means	986.9 ± 5.06	987.4 ± 5.06	

There was a significant enzyme x meal interaction for total bone mineral content of humeri from white-shelled egg laying hens (Table 5.5), however means were not found to be significantly different at either the 0.05 or 0.10 level (Table 5.6). Cortical BMC means were not different from one another ($P > 0.05$, Table 5.5). The overall means for total and cortical BMC were 8.47 and 11.42 mg/mm (Table 5.6), respectively.

Table 5.5. ANOVA *P*-values for humerus bone mineral content measurements from white-shell egg laying hens

	Bone Mineral Content	
	Total	Cortical
Source of Variation		
Enzyme	0.1699	0.6007
Meal	0.8052	0.8032
Enzyme*Meal	0.0308	0.0761

Table 5.6. Humerus bone mineral content measurements (mg/mm) from white-shell egg laying hens

Total Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	7.33 ± 1.046	9.12 ± 1.046	8.18 ± 0.740
10 % Canola Meal	9.47 ± 1.046	6.68 ± 1.046	7.95 ± 0.740
20 % Canola Meal	8.06 ± 1.046	8.99 ± 1.046	8.51 ± 0.740
10 % Juncea Meal	10.06 ± 1.046	6.80 ± 1.046	8.27 ± 0.740
20 % Juncea Meal	9.50 ± 1.046	8.71 ± 1.046	9.09 ± 0.740
Enzyme Means	8.82 ± 0.468	7.99 ± 0.468	
Cortical Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	10.72 ± 0.471	11.86 ± 0.471	11.29 ± 0.333
10 % Canola Meal	12.16 ± 0.471	10.60 ± 0.471	11.38 ± 0.333
20 % Canola Meal	11.66 ± 0.471	11.51 ± 0.471	11.59 ± 0.333
10 % Juncea Meal	11.93 ± 0.471	11.44 ± 0.471	11.69 ± 0.333
20 % Juncea Meal	11.03 ± 0.471	11.30 ± 0.471	11.17 ± 0.333
Enzyme Means	11.50 ± 0.211	11.34 ± 0.211	

There were no values for trabecular density or bone mineral content (Tables 5.3-5.6) because the majority of bones had a value of zero for density and BMC. However, during QCT scanning, 12 humeri were found to have bone in the trabecular space. These densities varied greatly (Table 5.7).

Table 5.7. Humeri of white-shell egg laying hens with medullary bone as indicated by bone in the trabecular space

Cage	Meal	Enzyme	Density Values (mg/cm^3)
39	10 % Canola	No	44.7
100	10 % Canola	No	48.0
35	20 % Canola	No	88.5
30	10 % Juncea	No	65.7
44	10 % Juncea	No	50.4
44	10 % Juncea	No	15.8
36	20 % Juncea	No	102.4
58	20 % Juncea	No	115.4
40	Soybean Meal	Yes	98.0
48	Soybean Meal	Yes	8.5
34	20 % Canola	Yes	96.7
93	20 % Juncea	Yes	3.7

Humeri from hens consuming enzymes had marginally significant lower (Table 5.8) bone weights than hens which were not provided with enzyme (Table 5.9). There was an enzyme x meal interaction (Table 5.8) for bone length, where 10 % CM+E had a lower bone length than 10 % CM-E (Table 5.9). There were no significant effects on bone width or bone breaking strength (Table 5.8) which averaged 6.8 mm and 11.95 kg Force, respectively (Table 5.9).

Table 5.8. ANOVA *P*-values for humerus bone quality measurements from white-shell egg laying hens

	Bone Quality Measurement			
	Weight	Length	Width	Breaking Strength
Source of Variation				
Enzyme	0.0583	0.0083	0.5616	0.6642
Meal	0.7221	0.5775	0.6397	0.6386
Enzyme*Meal	0.3963	0.0096	0.2470	0.5045

Table 5.9. Humerus bone quality measurements from white-shell egg laying hens

Bone Weight (g)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	4.7 ± 0.24	4.9 ± 0.24	4.8 ± 0.17
10 % Canola Meal	4.8 ± 0.24	4.3 ± 0.24	4.5 ± 0.17
20 % Canola Meal	5.0 ± 0.24	4.3 ± 0.24	4.6 ± 0.17
10 % Juncea Meal	5.0 ± 0.24	4.6 ± 0.24	4.8 ± 0.17
20 % Juncea Meal	4.8 ± 0.24	4.7 ± 0.24	4.7 ± 0.17
Enzyme Means	**4.8 [a] ± 0.11**	**4.5 [b] ± 0.11**	

Length (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	**72 [ab] ± 0.7**	**74 [ab] ± 0.7**	73 ± 0.5
10 % Canola Meal	**75 [a] ± 0.7**	**72 [b] ± 0.7**	73 ± 0.5
20 % Canola Meal	**75 [a] ± 0.7**	**73 [ab] ± 0.7**	74 ± 0.5
10 % Juncea Meal	**74 [ab] ± 0.7**	**73 [ab] ± 0.7**	73 ± 0.5
20 % Juncea Meal	**73 [ab] ± 0.7**	**73 [ab] ± 0.7**	73 ± 0.5
Enzyme Means	74 ± 0.3	73 ± 0.3	

Width (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	6.5 ± 0.30	6.8 ± 0.30	6.7 ± 0.22
10 % Canola Meal	7.3 ± 0.30	6.3 ± 0.30	6.8 ± 0.22
20 % Canola Meal	6.9 ± 0.33	7.3 ± 0.33	7.0 ± 0.23
10 % Juncea Meal	6.8 ± 0.33	6.5 ± 0.30	6.6 ± 0.23
20 % Juncea Meal	6.7 ± 0.30	6.7 ± 0.30	6.7 ± 0.22
Enzyme Means	6.8 ± 0.14	6.7 ± 0.14	

Breaking Strength (kg Force)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	11.89 ± 0.922	11.90 ± 0. 922	11.90 ± 0.652
10 % Canola Meal	12.62 ± 0. 922	10.41 ± 0. 922	11.49 ± 0. 652
20 % Canola Meal	12.26 ± 0. 922	13.31 ± 0. 922	12.78 ± 0. 652
10 % Juncea Meal	11.97 ± 0. 922	12.12 ± 0. 922	12.05 ± 0. 652
20 % Juncea Meal	11.59 ± 0. 922	11.39 ± 0. 922	11.49 ± 0. 652
Enzyme Means	12.07 ± 0.412	11.81 ± 0.412	

a-b means ± SEM within interaction or main effects with different superscripts are significantly different $\alpha \leq 0.05$

Correlations were performed on bone quality measurements and egg shell-quality measurements. Cortical density of humeri from white-shelled egg laying hens was found to have a significant, weak, inverse relationship with shell breaking strength (Table 5.10).

Table 5.10. Correlation coefficients (r) of humerus measurements with egg-shell measurements of white-shell egg laying hens

	Egg Measurements		
Humerus Measurement	Shell Weight	Shell Breaking Strength	
Area			
Total	0.0763	0.1541	
Cortical	0.0533	-0.1213	
Trabecular	0.0724	0.1685	
Density			
Total	-0.0358	-0.2032	
Cortical	0.1517	-0.2927	*
Bone Mineral Content			
Total	-0.0179	-0.1863	
Cortical	0.0872	-0.1802	
Bone Quality			
Weight	0.1569	0.1139	
Breaking Strength	0.0638	-0.0223	

*Correlations are significant with $\alpha \leq 0.05$

5.6.2 Tibia Measurements for White-Shell Egg Laying Hens

Enzyme supplementation of diets for white-shell egg laying hens resulted in a significantly lower (Table 5.11) total and cortical cross-sectional area of tibias (Table 5.12). Enzyme supplementation had a significant effect (Table 5.11) on trabecular area, where trabecular area increased by 1.94 mm^2 when enzyme was included in the diet (Table 5.12). There was no interaction effect, or effect of meal ($P > 0.05$, Table 5.11) on the tibia cross-sectional area.

Significantly greater (Table 5.13) cortical and trabecular density values were found for hens which received dietary enzyme supplementation (Table 5.14). There was no treatment effect on total density ($P > 0.05$, Table 13) but the overall mean was 663.2 mg/cm^3 (Table 5.14).

Table 5.11. ANOVA *P*-values for tibia cross-sectional area measurements from white-shell egg laying hens

	Area		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.0350	0.0029	0.0494
Meal	0.7710	0.9000	0.1585
Enzyme*Meal	0.1826	0.3433	0.1535

Table 5.12. Tibia cross-sectional area measurements for white-shell egg laying hens

Total Area (mm^2)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	39.97 ± 1.042	41.00 ± 1.042	40.49 ± 0.737
10 % Canola Meal	41.47 ± 1.042	37.40 ± 1.042	39.43 ± 0.737
20 % Canola Meal	40.97 ± 1.042	40.38 ± 1.042	40.68 ± 0.737
10 % Juncea Meal	40.86 ± 1.042	39.04 ± 1.042	39.95 ± 0.737
20 % Juncea Meal	40.75 ± 1.042	39.10 ± 1.042	39.93 ± 0.737
Enzyme Means	**40.80^{a} ± 0.466**	**39.39^{b} ± 0.466**	
Cortical Area (mm^2)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	20.24 ± 1.275	19.32 ± 1.275	19.76 ± 0.901
10 % Canola Meal	21.04 ± 1.275	18.86 ± 1.275	19.88 ± 0.901
20 % Canola Meal	20.04 ± 1.275	20.16 ± 1.275	20.12 ± 0.901
10 % Juncea Meal	21.22 ± 1.275	18.51 ± 1.275	19.73 ± 0.901
20 % Juncea Meal	22.70 ± 1.275	18.93 ± 1.275	20.58 ± 0.901
Enzyme Means	**20.99^{a} ± 0.570**	**19.14^{b} ± 0.570**	
Trabecular Area (mm^2)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	17.41 ± 1.723	21.47 ± 1.723	19.54 ± 1.218
10 % Canola Meal	18.47 ± 1.723	17.09 ± 1.723	17.79 ± 1.218
20 % Canola Meal	19.59 ± 1.723	18.72 ± 1.723	19.16 ± 1.218
10 % Juncea Meal	16.24 ± 1.723	19.51 ± 1.723	17.95 ± 1.218
20 % Juncea Meal	13.10 ± 1.723	18.18 ± 1.723	15.85 ± 1.218
Enzyme Means	**17.11^{b} ± 0.770**	**19.05^{a} ± 0.770**	

a-b enzyme means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Table 5.13. ANOVA *P*-values for tibia density measurements from white-shell egg laying hens			
	Density		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.2521	0.0101	0.0082
Meal	0.5973	0.3473	0.1910
Enzyme*Meal	0.6748	0.1179	0.1044

Table 5.14. Tibia density measurements (mg/cm^3) for white-shell egg laying hens

	Total Density (mg/cm^3)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	665.5 ± 22.53	634.0 ± 22.53	649.8 ± 15.93
10 % Canola Meal	687.6 ± 22.53	667.6 ± 22.53	677.6 ± 15.93
20 % Canola Meal	650.8 ± 22.53	677.2 ± 22.53	664.0 ± 15.93
10 % Juncea Meal	664.9 ± 22.53	634.7 ± 22.53	649.8 ± 15.93
20 % Juncea Meal	688.5 ± 22.53	661.6 ± 22.53	675.0 ± 15.93
Enzyme Means	671.5 ± 10.08	655.0 ± 10.08	
	Cortical Density (mg/cm^3)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	1057.2 ± 16.55	1091.9 ± 16.55	1074.5 ± 11.70
10 % Canola Meal	1068.0 ± 16.55	1062.9 ± 16.55	1065.5 ± 11.70
20 % Canola Meal	1073.8 ± 16.55	1070.5 ± 16.55	1072.1 ± 11.70
10 % Juncea Meal	1029.6 ± 16.55	1069.8 ± 16.55	1049.7 ± 11.70
20 % Juncea Meal	1012.0 ± 16.55	1083.8 ± 16.55	1047.9 ± 11.70
Enzyme Means	**1048.1^{b} ± 7.40**	**1075.8^{a} ± 7.40**	
	Trabecular Density (mg/cm^3)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	232.0 ± 14.28	223.3 ± 14.28	227.7 ± 10.10
10 % Canola Meal	230.9 ± 14.28	232.5 ± 14.28	231.7 ± 10.10
20 % Canola Meal	223.9 ± 14.28	269.0 ± 14.28	247.4 ± 10.10
10 % Juncea Meal	205.0 ± 14.28	229.7 ± 14.28	217.7 ± 10.10
20 % Juncea Meal	193.1 ± 14.28	245.4 ± 14.28	220.8 ± 10.10
Enzyme Means	**217.5^{b} ± 6.39**	**240.5^{a} ± 6.39**	

a-b enzyme means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Enzyme supplementation also had an effect on tibia total, cortical and trabecular BMC for white-shell egg laying hens (Table 5.15). Both total and cortical BMC were lower for hens without dietary enzyme than hens which were fed supplemental enzyme (Table 5.16). Trabecular BMC was 0.72 mg/mm greater with dietary enzyme (Table 5.16).

Table 5.15. ANOVA *P*-values for tibia bone mineral content measurements from white-shell egg laying hens

	Bone Mineral Content		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.0132	0.0090	0.0027
Meal	0.8433	0.9184	0.0831
Enzyme*Meal	0.2801	0.3823	0.1188

Similar to the results for humeri from white-shelled egg laying hens, tibia weight was significantly (Table 5.17) less with the addition of dietary enzyme compared to hens that were not fed the enzyme (Table 5.18). There was a significant enzyme x meal effect (Table 5.17) on bone width. SBM-E had a greater bone width than 10 % CM-E (Table 5.18). There were no significant treatment effects on bone length or breaking strength (Table 5.17) and values averaged 121 mm and 24.31 kg force, respectively (Table 5.18).

Table 5.16. Tibia bone mineral content measurements (mg/mm) for white-shell egg laying hens

	Total Bone Mineral Content (mg/mm)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	26.57 ± 1.016	25.94 ± 1.016	26.25 ± 0.718
10 % Canola Meal	28.46 ± 1.016	24.92 ± 1.016	26.69 ± 0.718
20 % Canola Meal	26.61 ± 1.016	27.23 ± 1.016	26.92 ± 0.718
10 % Juncea Meal	27.21 ± 1.016	24.77 ± 1.016	25.99 ± 0.718
20 % Juncea Meal	28.05 ± 1.016	25.87 ± 1.016	26.96 ± 0.718
Enzyme Means	**27.38[a] ± 0.454**	**25.75[b] ± 0.454**	
	Cortical Bone Mineral Content (mg/mm)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	21.48 ± 1.101	21.18 ± 1.101	21.33 ± 0.778
10 % Canola Meal	22.93 ± 1.101	20.27 ± 1.101	21.51 ± 0.778
20 % Canola Meal	21.69 ± 1.101	21.78 ± 1.101	21.73 ± 0.778
10 % Juncea Meal	22.46 ± 1.101	19.86 ± 1.101	21.08 ± 0.778
20 % Juncea Meal	23.49 ± 1.101	20.58 ± 1.101	21.94 ± 0.778
Enzyme Means	**22.38[a] ± 0.492**	**20.71[b] ± 0.492**	
	Trabecular Bone Mineral Content (mg/mm)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	4.16 ± 0.430	4.75 ± 0.430	4.46 ± 0.304
10 % Canola Meal	4.40 ± 0.430	4.07 ± 0.430	4.24 ± 0.304
20 % Canola Meal	4.25 ± 0.430	4.82 ± 0.430	4.54 ± 0.304
10 % Juncea Meal	3.26 ± 0.430	4.43 ± 0.430	3.89 ± 0.304
20 % Juncea Meal	2.57 ± 0.430	4.46 ± 0.430	3.64 ± 0.304
Enzyme Means	**3.79[b] ± 0.192**	**4.51[a] ± 0.192**	

a-b enzyme means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Table 5.17. ANOVA *P*-values for tibia bone quality measurements from white-shell egg laying hens

	Bone Quality Measurement			
	Weight	Length	Width	Breaking Strength
Source of Variation				
Enzyme	0.0431	0.1046	0.6247	0.2225
Meal	0.6491	0.6007	0.5015	0.7565
Enzyme*Meal	0.4311	0.7824	0.0165	0.6932

Table 5.18. Tibia bone quality measurements from white-shell egg laying hens

Bone Weight (g)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	11.1 ± 0.58	10.8 ± 0.58	10.9 ± 0.41
10 % Canola Meal	12.1 ± 0.58	9.9 ± 0.58	11.0 ± 0.41
20 % Canola Meal	11.2 ± 0.58	10.7 ± 0.58	11.0 ± 0.41
10 % Juncea Meal	10.8 ± 0.58	10.6 ± 0.58	10.7 ± 0.41
20 % Juncea Meal	10.5 ± 0.58	9.9 ± 0.58	10.2 ± 0.41
Enzyme Means	**11.1[a] ± 0.26**	**10.4[b] ± 0.26**	

Length (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	122 ± 1.5	121 ± 1.5	122 ± 1.1
10 % Canola Meal	124 ± 1.5	120 ± 1.5	122 ± 1.1
20 % Canola Meal	122 ± 1.5	122 ± 1.5	122 ± 1.1
10 % Juncea Meal	121 ± 1.5	120 ± 1.5	121 ± 1.1
20 % Juncea Meal	121 ± 1.5	119 ± 1.5	120 ± 1.1
Enzyme Means	122 ± 0.7	121 ± 0.7	

Width (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	**6.9 [ab] ± 0.24**	**7.6 [a] ± 0.24**	7.3 ± 0.17
10 % Canola Meal	**7.4 [ab] ± 0.24**	**6.5 [b] ± 0.24**	6.9 ± 0.17
20 % Canola Meal	**7.1 [ab] ± 0.24**	**7.4 [ab] ± 0.24**	7.3 ± 0.17
10 % Juncea Meal	**7.0 [ab] ± 0.24**	**6.9 [ab] ± 0.24**	6.3 ± 0.17
20 % Juncea Meal	**7.3 [ab] ± 0.24**	**6.9 [ab] ± 0.24**	7.1 ± 0.17
Enzyme Means	7.1 ± 0.11	7.1 ± 0.11	

Breaking Strength (kg Force)			
	Enzyme		
	No	No	Meal Means
Meal			
Soybean Meal	23.41 ± 1.722	24.35 ± 1.722	23.88 ± 1.218
10 % Canola Meal	26.13 ± 1.722	22.74 ± 1.722	24.43 ± 1. 218
20 % Canola Meal	25.38 ± 1.722	25.41 ± 1.722	25.39 ± 1. 218
10 % Juncea Meal	24.54 ± 1.722	21.81 ± 1.722	23.17 ± 1. 218
20 % Juncea Meal	25.46 ± 1.722	23.89 ± 1.722	24.68 ± 1. 218
Enzyme Means	24.98 ± 0.770	23.64 ± 0.770	

a-b means ± SEM within interaction or main effects with different superscripts are significantly different $\alpha \leq 0.05$

Correlations were performed on bone quality measurements and shell-quality measurements. Cortical density of tibias from WSLH had a significant ($P \leq 0.01$), weak, inverse relationship with shell breaking strength (Table 5.19). Similar relationships ($P \leq 0.05$) were found for trabecular density, total BMC and cortical BMC.

Table 5.19. Correlation coefficients (r) of tibia measurements with egg-shell measurements of white-shell egg laying hens

	Egg Measurements		
Tibia Measurement	Shell Weight	Shell Breaking Strength	
Area			
Total	-0.1484	0.0386	
Cortical	0.0987	-0.2479	
Trabecular	-0.1047	0.1922	
Density			
Total	0.1135	-0.3427	**
Cortical	-0.0452	0.1161	
Trabecular	-0.1455	-0.3325	*
Bone Mineral Content			
Total	0.0503	-0.2956	*
Cortical	0.1617	-0.3012	*
Trabecular	-0.1661	0.0803	
Bone Quality			
Weight	-0.1041	0.0074	
Breaking Strength	0.0830	-0.1854	

*Correlations are significant with $\alpha \leq 0.05$, **Correlations are significant with $\alpha \leq 0.01$

5.6.3 Humerus Measurements for Brown-Shell Egg Laying Hens

All the same measurements were taken for humeri of BSLH as for WSLH, however no significant differences were found for any of the variables measured on humeri from BSLH. For means of humeri variables measured see Appendix G.

Total BMC data was transformed using a square root transformation. Normality was achieved, but no significant correlations were found for this variable. Cortical BMC of humeri from brown-shell egg laying hens had a significant, positive, weak relationship with egg-shell breaking strength when correlations were performed (Table 5.20).

Table 5.20. Correlation coefficients (r) of humerus measurements with egg-shell measurements of brown-shell egg laying hens		
	Egg Measurements	
Humerus Measurement	Shell Weight	Shell Breaking Strength
Area		
Total	0.0387	0.1886
Cortical	-0.0931	0.0796
Trabecular	0.0693	0.1755
Density		
Total	-0.0823	0.1019
Cortical	-0.0492	-0.0013
Bone Mineral Content		
Total	-0.0599	0.1417
Cortical	0.0579	0.2999 *
Bone Quality		
Weight	0.1338	0.0342
Breaking Strength	0.0422	0.0494

*Correlations are significant with $\alpha \leq 0.05$

5.6.4 Tibia Measurements for Brown-Shell Egg Laying Hens

All the same measurements were taken for tibia of BSLH as for WSLH, however no significant differences were found for the majority of the variables measured on tibias from BSLH. Only those variables with significant treatment differences were reported in this chapter. For means of tibia variables measured with no significant differences due to treatment, see Appendix H.

Table 5.21. ANOVA *P*-values for tibia bone quality measurements from brown-shell egg laying hens				
	Bone Quality Measurement			
	Weight	Length	Width	Breaking Strength
Source of Variation				
Enzyme	0.3900	0.9743	0.8792	0.6941
Meal	0.5422	0.4962	0.1684	0.3684
Enzyme*Meal	0.0703	0.0191	0.5425	0.4768

Bone length had a significant meal x enzyme interaction (Table 5.21), but no differences could be detected for the least squares means at either the 0.05 or 0.10 level (Table 5.22). The overall mean for bone length was 122 mm.

Table 5.22. Tibia bone quality measurements from brown-shell egg laying hens

Bone Weight (g)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	14.2 ± 0.73	12.1 ± 0.73	13.2 ± 0.52
10 % Canola Meal	13.4 ± 0.73	11.3 ± 0.73	12.4 ± 0.52
20 % Canola Meal	12.5 ± 0.73	13.2 ± 0.73	12.9 ± 0.52
10 % Juncea Meal	12.4 ± 0.73	13.5 ± 0.73	13.0 ± 0.52
20 % Juncea Meal	11.8 ± 0.73	12.3 ± 0.73	12.1 ± 0.52
Enzyme Means	12.9 ± 0.33	12.5 ± 0.33	
Length (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	122 ± 1.2	122 ± 1.2	122 ± 0.9
10 % Canola Meal	124 ± 1.2	119 ± 1.2	121 ± 0.9
20 % Canola Meal	120 ± 1.2	124 ± 1.2	121 ± 0.9
10 % Juncea Meal	121 ± 1.2	122 ± 1.2	122 ± 0.9
20 % Juncea Meal	121 ± 1.2	120 ± 1.2	120 ± 0.9
Enzyme Means	121 ± 0.5	121 ± 0.5	
Width (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	8.4 ± 0.26	8.4 ± 0.26	8.4 ± 0.18
10 % Canola Meal	7.6 ± 0.26	8.0 ± 0.26	7.8 ± 0.18
20 % Canola Meal	7.9 ± 0.26	7.8 ± 0.26	7.8± 0.18
10 % Juncea Meal	7.9 ± 0.26	8.0 ± 0.26	7.9 ± 0.18
20 % Juncea Meal	8.1 ± 0.26	7.6 ± 0.26	7.9 ± 0.18
Enzyme Means	8.0 ± 0.12	8.0 ± 0.12	
Breaking Strength (kg Force)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	23.03 ± 1.933	20.36 ± 1.933	21.70 ± 1.367
10 % Canola Meal	23.76 ± 1.933	20.56 ± 1.933	22.16 ± 1.367
20 % Canola Meal	22.83 ± 1.933	21.33 ± 1.933	22.08 ± 1.367
10 % Juncea Meal	20.94 ± 1.933	19.47 ± 1.933	20.21 ± 1.367
20 % Juncea Meal	21.34 ± 1.933	24.64 ± 1.933	22.99 ± 1.367
Enzyme Means	22.38 ± 0.865	21.27 ± 0.865	

There were no significant differences for bone weight, width, or breaking strength (Table 5.21) and values averaged 12.7 g, 8.0 mm and 22.03 kg force (Table 5.22), respectively.

Tibia total BMC and breaking strength were positively (weakly) correlated with egg specific gravity (Table 5.23) when correlations were performed.

Table 5.23. Correlation coefficients (r) of tibia measurements with egg-shell measurements of brown-shell egg laying hens

	Egg Measurements			
Tibia Measurement	Specific Gravity		Shell Weight	Shell Breaking Strength
Area				
Total	0.0998		-0.0748	-0.2111
Cortical	-		-	-
Trabecular	0.0035		-0.1027	-0.1111
Density				
Total	0.1866		0.1689	0.0905
Cortical	0.0194		-0.0094	0.0699
Trabecular	0.0887		-0.0638	-0.1118
Bone Mineral Content				
Total	0.2511	*	0.1041	-0.0650
Cortical	0.0731		0.0969	-0.1211
Trabecular	0.0700		-0.0566	-0.1150
Bone Quality				
Weight	0.0360		-0.0751	-0.0559
Length	0.0825		-0.1144	-0.0843
Breaking Strength	0.2529	*	0.2105	0.0988

*Correlations are significant with $\alpha \leq 0.05$

5.7 Discussion

5.7.1 Humerus Measurements for White- and Brown-Shell Egg Laying Hens

When hens enter the laying period, formation of structural bone ceases while formation of medullary bone begins. Calcium for shell formation is supplied by medullary bone when dietary Ca levels are inadequate, such as during a dark peiod when hens are not consuming feed (Whitehead 2004). This results in daily synthesis and resorption of medullary bone (Candlish 1971). However, medullary bone is not normally present in the trabecular space of the humerus of poultry, so any bone resorption that occurred in the humerus would have been of cortical bone (Whitehead 2004). A decrease

in total cross-sectional area, such as the one in the 20 % JM-E treatments compared to the 10 and 20 % CM-E treatments (Table 5.2) could be explained by this. The osteoclasts that are responsible for bone resorption do not specifically target medullary bone, and would resorb structural bone if it was exposed.

This could also explain the weak, inverse relationship between cortical density and shell breaking strength for WSHL (Table 5.10). If osteoclasts were resorbing some cortical bone from the humeri (causing a decrease in cortical bone density) an increase in shell strength would be expected because the Ca and P freed from the bone would have been deposited in the egg-shell.

This does not explain the weak positive relationship between cortical BMC and shell breaking strength for BSLH (Table 5.19). This relationship indicated that as the BMC increased, so did the egg-shell breaking strength, which was not expected.

The 10 and 20 % CM-E treatments had a total cross-sectional area that was 6.6 mm^2 larger than the 20 % JM-E treatment. However, neither the 10 and 20 % CM-E treatments nor the 20 % JM-E treatment were different from any of the other treatments, especially the SBM-E control. This indicated that both diets could be used to replace some of the SBM if one or the other was available. However, if both were available, the CM should be included as it performed better than the JM, without dietary enzyme.

No previous research has reported the effects of CM, JM or enzyme supplementation on humerus quality measurements of laying hens. However, other studies have reported these measurements for commercial white-shell egg laying hens. The values obtained for total (49.22 to 55.82 mm^2) and trabecular (37.57 to 43.11 mm^2) cross-sectional area (Table 5.2) were higher than those obtained in other studies (approximately 40 and 29 mm^2, respectively) with commercial white-shell laying hens at similar ages (Riczu et al 2004, Silversides et al. 2006a and Jendral et al. 2008). Cortical area values (10.78 to 12.15 mm^2) were similar to those found by Silversides et al. (2006a, 11.92 mm^2) but were higher than those found by Riczu et al. (2004, 9.29 mm^2) and Jendral et al. (2008, 9.30 mm^2).

Analysis of variance found a marginally significant enzyme x meal interaction for total bone density (Table 5.3) and BMC (Table 5.5), but when the Tukey-Kramer method was used to compare the differences among the least-squares means, no significant

differences could be detected (Table 5.4 and Table 5.6). This may have been because interactions can be affected by the amount of data analyzed. In this analysis there were 5 dietary combinations plus 2 enzyme levels, which resulted in 10 total dietary treatments. Tukey-Kramer controls the type I error for the entire experiment (experimental error) as opposed to a less conservative test like LSD, which controls type one error for the comparison only. With LSD, when you set an α-value (ie. $P = 0.05$), this is the value tested. However, with the Tukey-Kramer method each comparison made slightly reduced the α-value tested. With 10 dietary treatments, there were 5 possible comparison pairs, each of which reduced the alpha value. So as the number of pairs increased, the probability of seeing differences due to treatment decreased, which may explain why ANOVA recognized treatment differences that were not detected at either the 0.1 or 0.05 level during comparison of least squares means.

The means for total density ranged from 134.9 to 201.2 mg/cm^3 (Table 5.4), which were similar to values found in other studies with commercial white-shelled laying hens at the end of the laying cycle (Riczu et al. 2004 and Jendral et al. 2008). Cortical density ranged from 959.4 to 1005.7 mg/cm^3 (Table 5.4). These values were similar to those found in some studies (963.3 mg/cm^3 and 1024 mg/cm^3 by Riczu et al. 2004 and Silversides et al. 2006b, respectively), but were lower than those found in other, similar studies (1125 mg/cm^3 and 1048 mg/cm^3 by Silversides et al. 2006a and Jendral et al. 2008, respectively).

Means for total (6.68 to 10.06 mg/mm, Table 6) and cortical (10.60 to 12.16 mg/mm, Table 5.6) BMC were similar to those found by Jendral et al. (2008), who found a total BMC of 6.07 mg/mm and a cortical BMC of 9.59 mg/mm for hens ages 65 weeks, housed in conventional battery cages.

Although humeri are hollow, pneumatic bones, and do not tend to have bone in the trabecular space, 12 of the 80 humeri measured were found to contain medullary bone in the trabecular space (Table 5.7). Fleming et al. (1996) found up to 50 % of a flock could have humeri with some medullary bone content at the end of the laying period. While no studies using CM or JM have measured the amount of medullary bone in the trabecular space of humeri, studies with commercial WSLH have. Each study had one

humerus with medullary bone in the trabecular space with densities of 4.21 mg/cm^3 (Riczu et al. 2004) and 84 mg/cm^3 (Silversides et al. 2006b).

Enzyme supplementation had a marginally significant effect on humerus weight, where humerus weight decreased (Table 5.9). No previous research has evaluated the effects of enzyme supplementation on laying hen humerus weight. One study evaluated tibia weight in broilers and found an increase with phytase supplementation (van der Klis et al. 1997) while another study found that there was no significant effect (Czerwiński et al. 2012).

There was an enzyme x meal interaction (Table 5.9) where hens fed 10 % CM-E had a greater humeral length (75 mm) than hens fed 10 % CM+E (72 mm). This was not expected because bone formation and growth occur before the onset of sexual maturity in laying hens (Whitehead 2004 and Fleming 2008). After the onset of lay, medullary bone is formed and resorbed for egg shell production, but no structural bone is produced until the hen stops laying eggs. The hens in the current study did not receive the test diets until 30 weeks of age, and the usual age of sexual maturity in laying hens (marked by the onset of lay) is 20 weeks of age. Therefore, the difference in bone length may have been present since the beginning of the trial, but was not detected as bone measurements were only taken once. Hester et al. (2011) found the average humeral length of white-shell egg laying hens at 66 weeks of age to be 72.4 mm, which was close to the values found in the current study.

As expected, there were no significant differences found among treatments for humeral width. The values ranged from 6 to 7 mm (Table 5.9), and were similar to humeral widths (8.20 mm) found in a study using laying hens (Hester et al. 2011).

Values for humeral breaking strength (Table 5.9) were similar to those determined in several other studies with commercial WSLH (Fleming et al. 1994, Gordon and Roland 1997, Silversides et al. 2006a, and Jendral et al. 2008). Gordon and Roland (1997) included a phytase enzyme (which provided 300 phytase units/kg of feed) and found that there was no significant difference in humeral breaking strength, which concurs with the results from the current study.

5.7.2 Tibia Measurements for White- and Brown-Shell Egg Laying Hens

Enzyme supplementation caused a decrease in total and cortical area (Table 5.12), total and cortical BMC (Table 5.16) and bone weight (Table 5.18). It caused an increase in trabecular area (Table 5.12), density (Table 5.14) and BMC (Table 5.16) of tibias. Similar to the explanation for the changes in the humerus, the changes in the tibia can be explained by the resorption of bone for shell production. In this case, tibias do contain medullary bone. The increase in trabecular area, density and BMC were due to the replacement of medullary bone since medullary bone is classified as trabecular bone during QCT measurements (Korver et al. 2004).

There could still be a decrease in total, cortical and weight measurements because osteoclasts could have resorbed some of the structural bone, which would have been replaced with medullary bone. During shell formation some of the newly replaced medullary bone could be resorbed again, causing a decline in total and cortical area. If less medullary bone was replaced than was resorbed, there would be a net increase in trabecular area. A decline in area measurements would explain the decline found in BMC, because BMC was calculated by multiplying density and area. This decline in BMC further suggests that some bone was being resorbed for shell formation.

Total density, trabecular density and total BMC of WSLH were found to have weak, negative correlations with egg shell breaking strength (Table 5.19). This supports the theory that medullary bone was being mobilized from the tibia as a source of Ca for shell production. Humerus measurements of WSLH resulted in a weak, negative correlation between cortical density and shell-breaking strength (Table 5.10) which was not found for the tibias of these hens. This may be due to the fact that humeri do not generally contain medullary bone, so any osteoclast activity in this area would have resulted in cortical bone mobilization instead. Cortical BMC of tibias also had a weak, inverse relationship with egg-shell breaking strength (Table 5.19), suggesting that some cortical bone may also have been resorbed.

Bone resorption does not explain why there was a greater cortical density for hens which consumed the supplemental enzymes (Table 5.14). Perhaps enzyme supplementation protected the cortical bone in some way from resorption. Even though

there was a decline in area, the density of the remaining bone was not as affected as the cortical bone in tibias of hens which did not consume the enzymes.

Bone resorption does not explain the positive, weak correlations between tibia total BMC or bone breaking strength and egg-shell specific gravity of BSLH (Table 5.23). These are similar to the weak, positive correlation found for cortical BMC and shell breaking strength of humeri of BSLH (Table 5.20). Negative correlations were expected, because bone Ca and P is resorbed and deposited into the egg-shell, usually resulting in weaker bones and stronger shells. Egg-shell specific gravity is an indirect measure of shell strength (Roberts 2004). In this case however, it appears that as bones became stronger, so did the egg shells. This may suggest that for BSLH, enough Ca was being provided in the diet to allow medullary bone stores to be replenished. This is reinforced by the fact that there were no differences in QCT or bone quality measurements for BSLH.

No previous research has reported QCT measurements for white-shell laying hens consuming CM or JM. However, cortical area values for white-shell laying hens housed in conventional battery cages reported by Jendral et al. (2008) and Silversides et al. (2012) were similar (19.41 mm^2 and 20.70 mm^2, respectively) to those found in the current study (18.51 to 22.70 mm^2). The total and trabecular area values (31.81 and 9.45 mm^2, respectively) reported by Jendral et al. (2008) were lower than those found in the current study (37.40 to 41.47 mm^2 and 13.10 to 21.47 mm^2, respectively). Conversely, the total and trabecular area values (31.20 and 18.40 mm^2, respectively) reported by Silversides et al. (2012) were similar to those found in the current study.

Total density values (Jendral et al. 2008) were higher (735.98 mg/cm^3) than those found in the current study (634.0 to 688.5 mg/cm^3). However, cortical and trabecular densities were similar (1057.35 and 246.04 mg/cm^3, respectively) to those found in the current study (1012.0 to 1091.9 and 193.1 to 269.0 mg/cm^3, respectively). Silversides et al. (2012) reported total, cortical, and trabecular density values lower (608, 968 and 186 mg/cm^3, respectively) than those found in this study.

Jendral et al. (2008) reported BMC values for white-shell egg laying hens housed in conventional battery cages. Total, cortical and trabecular values were all similar (23.32, 19.36 and 2.40 mg/mm, respectively) to the low end of the range of values

reported in the current study (24.77 to 28.46, 19.86 to 23.49 and 2.57 to 4.82 mg/mm, respectively).

Enzyme supplementation resulted in a lower tibia weight than those hens which did not consume enzyme (10.4 g versus 11.1 g). This disagreed with results of a study that supplemented laying hen diets with up to 300 FTU/kg of basal diet and found no difference in weight of tibias (van der Klis et al. 1997). The tibia weights in the study done by van der Klis et al. (1997) ranged from 5.20 g to 5.38 g, but were measured on a fat-free, dry-matter basis. Nørgaard-Nielsen (1990) found that tibias of caged Danish commercial white leghorn laying hens weighed an average of 11.4 g. Silversides et al. (2012) reported an average tibia weight of 10.97 g. The tibia weights determined during the Danish study and by Silversides et al. (2012) were similar to tibia weights determined during the present study.

Tibia bone breaking strength was not affected by enzyme supplementation or meal type included in the diet. Means ranged from 22.74 to 26.13 kg Force and were lower than those reported by Nørgaard-Nielsen (1990), who found bone breaking strengths of 30.20 kg force. This study used commercial white-shell egg laying hens. These results differ from those reported by Czerwiński et al. (2012) who found that feeding supplemental phytase at 1000 FYT/kg of diet increased bone breaking strength in broiler chickens. No research has been reported which measures the effect of CM or JM on tibia breaking strength for laying hens.

QCT measurements for tibias of brown-shell egg laying hens have not been widely reported in previous literature. Therefore, there were no ‘normal’ values to compare with the QCT measurements obtained in the current study. However, bone quality measurements by Koutoulis et al. (2009) were similar to those obtained in the current study. Ranges of values for bone weight, length, width and breaking strength were 11.3 to 14.2 g, 119 to 124 mm, 8 mm and 19.47 to 24.64 kg force, respectively for the present study. These same measurements were found to be 11.61 to 11.93 g, 120 to 121 mm, 7 mm and 22.89 to 23.35 kg force, respectively by Koutoulis et al. (2009).

5.7.3 Combined Trial Discussion

Enzyme supplementation seemed to improve bone quality for WSLH by increasing trabecular cross-sectional area, density and BMC measurements. However, there was a decline in total and cortical area and density measurements, which may indicate that although enzyme supplementation could help rebuild medullary stores of bone it could not prevent the loss of structural bone. This was reinforced by the negative correlations found for humerus and tibia measurements with shell quality measurements. For brown-shell egg laying hens, no differences were found for QCT measurements, but positive correlations were seen between bone and shell quality measurements indicating that structural bone was protected from resorption and medullary bone stores were being adequately replenished.

Although there were significant correlations between bone and egg quality measurements, it is important to note that the correlation coefficients were very low. A correlation coefficient of approximately 3 (or -3) indicated that the relationship between the variables was very weak. A correlation between egg production or Ca output (number of eggs x average shell weight) and bone quality measurements may better explain what is truly occurring during the laying cycle.

Rennie et al. (1997) studied several different nutritional deficiencies that were suspected to delay or prevent osteoporosis (characterized by a general loss of skeletal bone mineral, Whitehead and Wilson 1992) caused by dietary Ca deficiency. They found osteoporosis did occur, but was not due to Ca deficiency, and could not be prevented by any of the nutritional treatments that were administered. Dietary treatments included feeding large particle oyster shell, 1,25-dihydroxycholecalciferol, ascorbic acid, fluoride, a reduced amount of phosphorus and a combination of a reduced amount of crude protein and higher amount of vitamin K.

Rennie et al. (1997) studied two genetically different strains of laying hen. One (J-line, a Brown Leghorn line) was maintained without selection while the other was highly selected (Hisex Brown) for production traits. This study indicated that the J-line hens were not osteoporotic at all, but a majority of the Hisex hens did develop osteoporosis. Since there were no effects of dietary treatment within strain of hen, Rennie et al. (1997) attributed the osteoporosis of the Hisex hens to the fact that they maintained

a high rate of production for an extended period of time. The J-line hens had a reduced rate of egg production when compared to the Hisex hens, which meant they would have entered and left periods of lay, allowing them time to rebuild the structural (cortical) bone reserves.

The differences between white- and brown- shell egg laying hens could be due to the fact that the Lohmann LSL-Lite white hens maintained a slightly higher level of egg production than the Lohmann Brown-Lite hens did (Section 3.6.2 and 3.6.3, respectively). This may have allowed the BSLH to rebuild some of the structural bone reserve before the end of the trial, which is why no enzyme effects were detected in these hens.

Similar to the dietary treatments in the study by Rennie et al. (1997), the supplemental enzymes used in the current study may not have been able to prevent the onset of osteoporosis in the white-shell egg laying hens. However, they were able to help increase the amount of medullary bone present in the tibia, which may help the hen maintain a longer period of egg production without utilizing cortical bone as a supply of Ca.

While enzyme may have helped to maintain medullary bone stores, and therefore, increased production, they could not prevent some structural bone loss. Therefore, enzymes should be used in combination with other bone management strategies, such as feeding large particle Ca sources (Saunders-Blades et al. 2009), feeding late in the photoperiod (Etches 1987), and allowing bone loading behaviours when possible (Fleming et al. 1994 and Jendral et al. 2008).

For future studies, bone measurements should be taken at more than one time period throughout the trial, to establish baseline bone values. This way, if a change attributed to treatment was detected, a magnitude of the change would be available. This may help to determine whether the change was actually detrimental to the overall health of the laying hen, or was a more natural fluctuation that occurs during egg shell formation.

5.8 Conclusions

The inclusion of 20 % juncea meal significantly reduced the total area measurement of humeri from white-shell egg laying hens, when compared with the 10 and 20 % canola meal diets, all without enzyme supplementation. However the 20 % juncea meal diet was not different from the soybean meal control. Tibia bone width of WSLH was reduced by 1 mm when hens were fed 10 % canola meal with enzyme compared to the soybean meal control with enzyme. The reduced tibia width (7 mm) was found to be within a normal range for WSLH. Finally, inclusion of CM and JM did not significantly affect any bone quality measurements for BSLH. Therefore the hypothesis was confirmed that up to 20 % CM and JM could be included in laying hen diets without significantly affecting bone characteristics.

The inclusion of dietary enzyme decreased bone weight, total cross-sectional area and BMC, and cortical cross-sectional area and BMC of white-shell egg laying hens. This was contrary to the original hypothesis. However, in past research, nutritional strategies were not enough to prevent the onset of osteoporosis, so the reduction in area and BMC measurements are within current normal ranges for laying hens. Inclusion of dietary enzyme did increase trabecular area, density and bone mineral content of tibias from WSLH. Since trabecular bone includes medullary bone in QCT measurements, and laying hens exclusively produce medullary bone during the laying cycle, this increase of trabecular bone is thought to be due to increased medullary bone deposition. This indicated that enzyme supplementation increased medullary bone reserves.

Inclusion of 10 % canola meal with enzyme supplementation decreased humeral length of WSLH compared to 10 % canola meal without enzyme. The reduced bone length (72 mm) was found to be within a normal range for WSLH. Although any reduction was contradictory to the original hypothesis, these values are within normal ranges for laying hens, and were therefore considered to be of no biological importance. Finally, bone breaking strength of WSLH and all bone measurements of BSLH were not affected by inclusion of dietary enzyme. Therefore dietary enzymes can be included in laying hen diets with no negative impact to bone quality, and may be used to increase tibia medullary bone reserves in white-shell egg laying hens.

Chapter 6. Effect of canola meal or juncea meal with or without supplemental dietary enzymes on liver health and mortality due to fatty liver hemorrhagic syndrome of white- and brown-shell egg laying hens.

6.1 Abstract

Fatty liver hemorrhagic syndrome (FLHS) is a disease in laying hens linked to the consumption of rapeseed and canola meals. There are often no outward symptoms of the disease until a sudden drop in egg production occurs, usually followed by an increase in flock mortality. FLHS can account for as much as 5 % of mortalities. Two trials compared the effects of canola meal (CM), juncea meal (JM) or soybean meal (SBM), with and without enzyme supplementation, on liver health and mortality due to FLHS. The trials were designed as 5x2 factorials in completely randomized design. A total of 360 Lohmann LSL-Lite White (Trial 1, WSLH) and 300 Lohmann Brown-Lite (Trial 2, BSLH) laying hens, housed in 60 cages, were fed one of 10 isoenergetic and isonitrogenous diets (SBM, 10 % CM, 20 % CM, 10 % JM or 20 % JM with or without a dietary enzyme cocktail of Superzyme OM^{TM} and Bio-Phytase^{TM}) for 48 weeks. At the end of the trial (approximately 78 weeks of age) 2 hens per cage were euthanized and livers were removed weighed, and measured for colour with a Hunter Lab Miniscan XE^{TM}. Livers were visually scored for colour, texture and hemorrhage score. Hepatosomatic index (HSI) was calculated any dry matter (DM) and fat content (% fat) were determined for each liver. For WHLS, neither meal type nor enzyme supplementation had a significant effect on any of the liver measurements ($P > 0.05$). For BSLH, enzyme supplementation had a marginally significant effect ($P = 0.0505$) on HSI where HSI increased from 1.78 to 1.87. Enzyme decreased b colour score (yellowness) from 23.83 to 22.18 ($P = 0.0390$) but had no effect on other liver measurements ($P > 0.05$). SBM resulted in a higher liver fat content (27.8 %) than either 10 % JM (22.7 %) or 20 % CM (22.0 %). Mortality due to fatty liver hemorrhagic syndrome (1.7 % for Trial 1 and 1.3 % for Trial 2) was not related to treatment. There is no evidence of liver damage related either to the use of CM or JM up to 20 %, or the use of enzymes in diets for WSLH or BSLH.

Key Words: *Canola, Juncea, Enzymes, Liver Characteristics, Poultry*

6.2 Introduction

Canola and juncea meals contain glucosinolates, which in the presence of the enzyme myrosinase, hydrolyse into breakdown products (Bones and Rossiter 1996). The breakdown products continue to react, forming compounds such as isothiocyanates (responsible for bitterness), and nitriles, thiocyanates, thiourea and oxazolidithione (Tripathi and Mishra 2007), which disrupt thyroid function by reducing the availability of iodine (Schöne et al. 1997). Glucosinolate metabolism can result in morphological and physiological changes to the thyroid, as well as impaired liver and kidney function (Tripathi and Mishra 2007).

Rapeseed meal has been shown to cause liver damage in laying hens and broiler chickens in the past, and has been shown to cause (Gough and Weber 1978) or modify (Pearson et al. 1978b) the symptoms of fatty liver hemorrhagic syndrome in laying hens. FLHS is a disease in laying hens that often has no symptoms until sudden death of a hen occurs. There may be some outward signs which include pale, enlarged combs, wattles covered with ‘dandruff’ (Harms et al. 1972) and an abrupt drop in egg production. Upon necropsy it becomes apparent that death was caused by a massive hemorrhage of the liver which ruptured the Gilsson’s capsule (Butler 1976). The liver itself is enlarged, pale yellowish-brown, has indications of smaller hemorrhages that did not rupture the Glisson’s capsule, and is extremely friable. The hen may also have a great deal of yellow abdominal fat in the body cavity and around the viscera (Fowler 1990).

Marangos and Hill (1976) found that feeding 12 % *Brassica napus* RSM to laying hens caused an increased liver weight and an increased mortality rate over hens fed a SBM control diet. The major cause of death for hens sent for necropsy was massive liver hemorrhage related to the consumption of RSM. This study determined that hens in full production seem to be more susceptible to liver hemorrhage when fed RSM than younger (growing) birds.

Although breeding has reduced the glucosinolate content of canola (from greater than 30 μmol/g in RSM to approximately 8-9 μmol/g in current canola cultivars) and juncea has been developed with a relatively low glucosinolate content (12.5 μmol/g), it still needs to be determined if the levels have been reduced enough to eliminate any negative impact on the liver of laying hens during breakdown product reactions.

6.3 Objectives

1. To determine the effect of canola meal or juncea meal included at 10 or 20 % of the diet on liver characteristics of white- and brown-shell egg laying hens including: weight, hepatosomatic index, colour, texture, and hemorrhage score, dry matter, fat content, and mortality rate due to FLHS.
2. To determine the effect of a supplemental dietary enzyme cocktail on liver characteristics of white- and brown-shell egg laying hens including: weight, hepatosomatic index, colour, texture, and hemorrhage score, dry matter, fat content, and mortality due to FLHS.

6.4 Hypothesis

The inclusion of canola or juncea meal will not have a significant effect on liver characteristics of white- or brown-shell egg laying hens including: weight, hepatosomatic index, colour, texture, or hemorrhage score, dry matter, fat content, or mortality due to FLHS. As well, there will be no significant effect of enzyme for any of the liver characteristics measured of white- or brown-shell egg laying hens.

6.5 Materials and Methods

6.5.1 Animals, Diets and Husbandry

Hens used to measure liver health and mortality due to FLHS were fed and cared for as described in Chapter 3. All birds were managed in accordance with the Dalhousie Agricultural Campus Animal Care and Use Committee guidelines that follow the Canadian Council on Animal Care Codes of Practice (2009).

6.5.2 Sample Collection and Analysis

At the end of each 48 week feeding trial, two hens per cage were euthanized by cervical dislocation. The livers of the euthanized hens were weighed and evaluated for colour, texture and prevalence of hemorrhage. Liver weight was related to hen body weight at the time of euthanasia to determine the heptasomatic index (HSI). Colour was evaluated using the Hunter Lab Miniscan XE^{TM} (Hunter Associates Laboratory, Inc., Reston, VA) with Colourimeter Colour using illuminant C and an observer of 2° (C/2°).

Measurements were based on the ‘L’, ‘a’, and ‘b’ scale where ‘L’ measures from dark to light, ‘a’ measures from green to red, and ‘b’ measures from blue to yellow (Fig. 6.1). Two readings for each liver were taken and averaged.

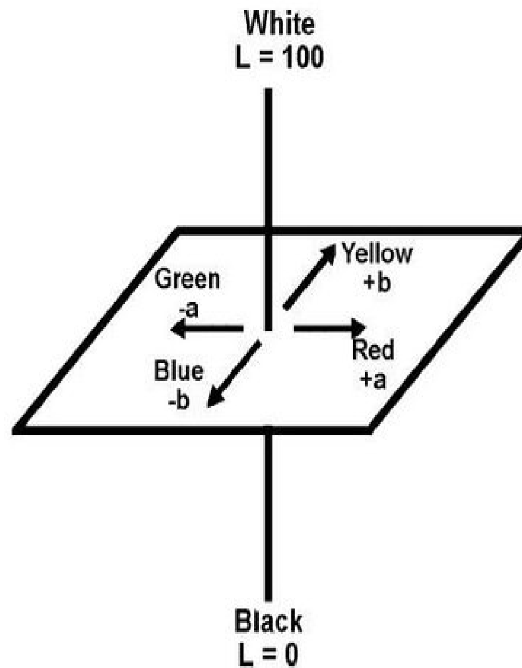

Fig. 6.8. ‘L’, ‘a’, ‘b’ colour scale from HunterLab (2008b).

The ‘L’, ‘a’, ‘b’ colour scale measures colour in a cube space. The ‘L’ axis measures lightness and ranges from 0 (black) to 100 (white). Positive values of ‘b’ are yellow while negative values are blue. Positive values of ‘a’ are red, while negative values are green. There are no numerical limits to the ‘a’ and ‘b’ axis, and values of zero are neutral.

Liver colour and texture were evaluated on a subjective scale modified from Guenter (W. Guenter, personal communication, University of Manitoba, Winnipeg, MB). The original scale ranked colour and texture together where the liver was rated 1 for dark and firm, 2 for pale and firm, 3 for dark and friable, and 4 for pale and friable. The modified scale separated colour and texture (Table 6.1), so that each would receive a score of 1 or 2. Texture was scored to give an indication of how fatty the liver was, as fatty livers were also found to be unusually soft (Wolford and Polin 1972 and Pearson et al. 1978a).

Table 6.1. Visual liver scoring scale for colour and texture		
	Characteristic	
Score	Colour	Texture
1	dark	firm
2	pale	friable

Livers were evaluated for hemorrhage based on a method modified from Diaz et al. (1999) and Guenter (W. Guenter, personal communication, University of Manitoba, Winnipeg, MB). Basically, the more descriptive scale from Diaz et al. (1999) was combined with the larger scale provided by Guenter to give a subjective scale that ranged from zero to five (Table 6.2). After visual scoring, the livers were individually placed in Whirl-Pak bags and were frozen at -20°C until further analysis could be conducted.

Table 6.2. Visual liver scoring scale for hemorrhage	
Score	Hemorrhages
0	No hemorrhages present
1	0 to 10 subcapsular petechial or ecchymotic
2	> 10 subcapsular petechial or ecchymotic
3	Hematomas present with diameter of ≤ 2 cm
4	Hematomas present with diameter of > 2 cm
5	Massive hemorrhage with rupture of Glisson's capsule

Dry matter (DM) analysis was carried out on the liver samples. They were removed from the -20°C freezer and allowed to return to room temperature. Livers were weighed in grams to two decimal places, and placed at -80°C overnight. Livers were freeze-dried in a MODULY0D freeze-dryer (ThremoFisher Scientific, Asheville, NC, USA) and then weighed again. After weighing, samples were ground and stored in labeled sample containers with screw tops.

Fat analysis was performed in duplicate on the freeze-dried liver samples using the Association of Official Analytical Chemists (AOAC) method 991.36 (AOAC 2005). One g of sample was weighed into pre-weighed bags, which were placed in tins that had been pre-dried for 15 minutes at 100°C and pre-weighed. The sample, bag, and tin were then oven dried for 3 hours in an Isotemp Drying Oven (Fisher Scientific, Ottawa, ON, Canada) at 100°C. After drying, samples were reweighed and the sample bags were placed in an ANKOM^{XT15} extraction system (Macedon, NY, USA) for one hour at 90°C

with petroleum ether as the solvent. After extraction, the sample bags were placed in an ANKOM[RD] drying oven (Macedon, NY, USA) for 30 minutes at 110°C then reweighed. Percent fat was calculated using the following equation:

$$\% fat = \frac{weight\ after\ extraction}{dry\ weight\ before\ extraction} x100\%$$

Finally, the duplicate percent fat values were checked for variation. If they were not within 3 % of each other, the analysis was repeated.

6.5.3 Statistical Analysis

All visual scoring measurements (texture, colour and hemorrhage score) were subjected to Chi-square (χ^2) analysis using 2-way contingency tables (Moore et al. 2009) with Minitab 16 software (Minitab, Inc., USA). For χ^2 analysis, each individual hen was used as the experimental unit. Meal type (soybean meal control, 10 % canola meal, 20 % canola meal, 10 % juncea meal and 20 % juncea meal) or supplemental enzyme (either present or absent) was used as the independent variables, while hemorrhage score, colour score or texture score was used as the dependent variables. Each dependent variable was tested for the effect of enzyme (2x2 tables for colour and texture, 2x6 tables for hemorrhage) or meal (5x2 tables for colour and texture, 5x6 tables for hemorrhage) in a separate table and therefore, enzyme by meal interactions were not accounted for in this analysis. For 2x2 contingency tables χ^2 was a good estimate of the distribution of colour or texture score when the expected count of all 4 cells were at least 5. For contingency tables larger than a 2x2, χ^2 was a good estimate of the distribution of colour, texture or hemorrhage score when the average of the expected counts were greater than 5 and when the smallest expected count was greater than or equal to 1. The α-level of significance was $P \leq 0.05$.

Both trials were completely randomized designs. Liver weight, HSI, ‘L’, ‘a’ and ‘b’ colour scores, DM, and fat content were subjected to Analysis of Variance (ANOVA) using the Proc Mixed procedure of the Statistical Analysis Systems (SAS), Inc. (Littell et al. 1996) with software version 9.3 (SAS Institute, Inc., Cary, NC, USA). Meal type and

supplemental enzyme were tested as the main effects. The statistical model used for liver measurements was:

$$Y_{ij} = \mu + \tau_i + \gamma_j + \tau\gamma_{ij} + \epsilon_{ijk}$$

Where Y_{ij} is the variable of interest; μ is the overall mean; τ_i is the effect of the ith meal (i = 1-5); γ_j is the effect of the jth dietary inclusion level of enzyme (j = 1-2); $\tau\gamma_{ij}$ is the effect of the interaction between meal and enzyme; and ϵ_{ijk} is the random effect of error, with k representing replicate cages (k = 1-6).

Repeated measures analysis was used for mortality data by adding the factor of time, δ_k (with production period as the measure of time, k = 1-12) and all resulting interaction levels with the main effect to the above model. For the random effect of error, l represented replicate cages. The following model was employed for repeated measures analysis:

$$Y_{ijk} = \mu + \tau_i + \gamma_j + \delta_k + \tau\gamma_{ij} + \tau\delta_{ik} + \gamma\delta_{jk} + \tau\gamma\delta_{ijk} + +\epsilon_{ijkl}$$

If significant main effects or interactions were found, the Tukey-Kramer procedure was used to compare differences among the least-square means. The standard error of each mean (SEM) was reported with the mean. The α-level of significance was $P \leq 0.05$.

6.6 Results

6.6.1 Liver Measurements for White-Shell Egg Laying Hens

Analysis of variance results for Hunter Lab colour scores (Table 6.3) showed that there was no treatment effect ($P > 0.05$) on liver colour. The values obtained averaged 34.47 for 'L' score, 16.13 for 'a' score and 22.95 for 'b' score (Table 6.4).

Table 6.3. ANOVA *P*-values for Hunter Lab colour scores of livers from white-shell egg laying hens

	Score		
	L	a	b
Source of Variation			
Enzyme	0.8135	0.9570	0.3685
Meal	0.9474	0.9104	0.8998
Enzyme*Meal	0.4721	0.6925	0.5476

Table 6.4. Hunter Lab colour scores of livers from white-shell egg laying hens

	L Score		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	34.20 ± 1.514	35.99 ± 1.514	35.10 ± 1.070
10 % Canola Meal	33.86 ± 1.514	35.39 ± 1.514	34.63 ± 1.070
20 % Canola Meal	35.18 ± 1.514	32.77 ± 1.514	33.98 ± 1.070
10 % Juncea Meal	33.72 ± 1.514	35.46 ± 1.514	34.59 ± 1.070
20 % Juncea Meal	34.84 ± 1.514	33.32 ± 1.514	34.08 ± 1.070
Enzyme Means	34.36 ± 0.677	34.58 ± 0.677	
	a Score		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	16.35 ± 0.762	15.82 ± 0.762	16.09 ± 0.539
10 % Canola Meal	16.43 ± 0.762	16.63 ± 0.762	16.53 ± 0.539
20 % Canola Meal	15.37 ± 0.762	16.56 ± 0.762	15.96 ± 0.539
10 % Juncea Meal	16.61 ± 0.762	15.59 ± 0.762	16.10 ± 0.539
20 % Juncea Meal	15.86 ± 0.762	16.05 ± 0.762	15.95 ± 0.539
Enzyme Means	16.12 ± 0.341	16.13 ± 0.341	
	b Score		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	22.03 ± 1.347	24.60 ± 1.347	23.31 ± 0.952
10 % Canola Meal	22.15 ± 1.347	24.54 ± 1.347	23.35 ± 0.952
20 % Canola Meal	22.58 ± 1.347	22.75 ± 1.347	22.66 ± 0.952
10 % Juncea Meal	23.16 ± 1.347	23.27 ± 1.347	23.21 ± 0.952
20 % Juncea Meal	22.92 ± 1.347	21.54 ± 1.347	22.23 ± 0.952
Enzyme Means	22.57 ± 0.602	23.34 ± 0.602	

ANOVA values for liver measurements (Table 6.5) indicated that there was no difference ($P > 0.05$) for any of the liver measurements taken in Trial 1. The overall means were 37.2 g for liver weight, 2.12 for HSI, 31.50 % for dry matter and 26.92 % for fat content (Table 6.6).

Table 6.5. ANOVA *P*-values for liver measurements of white-shell egg laying hens

	Liver Weight	Hepatosomatic Index	Dry Matter	Fat Content
Source of Variation				
Enzyme	0.1863	0.0696	0.8778	0.9763
Meal	0.0968	0.3265	0.1094	0.1370
Enzyme*Meal	0.8004	0.6159	0.8068	0.9373

Chi-Square analysis of hemorrhage score revealed that none of the livers received a score of zero (no hemorrhage) so this column was removed, reducing the 2-way contingency tables from a 5x6 for meal and 5x2 for enzyme, to a 4x6 and a 4x2 respectively. This however, did not change the final results, which indicated there was no meal or enzyme effect on hemorrhage score ($P > 0.05$, Table 6.7).

For χ^2 analysis of texture score, only one of the livers received a score of 1 (firm). This caused expected counts of less than 1 for both enzyme and meal effect, indicating that the χ^2 statistic was not a good estimate for the distribution of texture score. For this reason, χ^2 analysis could not be performed on this variable. However, because all livers but one received a score of 2 (friable), it is safe to say that there is no enzyme or meal effect on texture. While there is no treatment effect on texture, the condition of the liver tissue indicates that something may be occurring to weaken livers in the whole flock.

Chi-Square analysis of visual colour score also revealed that there was no meal or enzyme effect on this variable (Table 6.7).

Table 6.6. Liver measurements for white-shell egg laying hens

	Liver Weight (g)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	38.9 ± 2.37	38.5 ± 2.37	38.7 ± 1.68
10 % Canola Meal	39.1 ± 2.37	37.8 ± 2.37	38.5 ± 1.68
20 % Canola Meal	41.6 ± 2.37	36.6 ± 2.37	39.1 ± 1.68
10 % Juncea Meal	35.4 ± 2.37	33.7 ± 2.37	34.5 ± 1.68
20 % Juncea Meal	35.5 ± 2.37	35.0 ± 2.37	35.2 ± 1.68
Enzyme Means	38.1 ± 1.06	36.3 ± 1.06	
	Hepatosomatic Index (g liver / g body weight)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	2.18 ± 0.118	2.03 ± 0. 118	2.10 ± 0.084
10 % Canola Meal	2.12 ± 0. 118	2.22 ± 0. 118	2.17 ± 0.084
20 % Canola Meal	2.32 ± 0. 118	2.09 ± 0. 118	2.20 ± 0.084
10 % Juncea Meal	2.10 ± 0. 118	1.87 ± 0. 118	1.99 ± 0.084
20 % Juncea Meal	2.22 ± 0. 118	2.08 ± 0. 118	2.15 ± 0.084
Enzyme Means	2.19 ± 0.053	2.06 ± 0.053	
	Dry Matter (%)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	30.69 ± 1.061	31.91 ± 1.162	31.30 ± 0.787
10 % Canola Meal	33.40 ± 1.061	32.00 ± 1.061	32.70 ± 0.750
20 % Canola Meal	30.17 ± 1.161	30.66 ± 1.061	30.41 ± 0.787
10 % Juncea Meal	30.74 ± 1.061	30.48 ± 1.061	30.61 ± 0.750
20 % Juncea Meal	32.66 ± 1.061	32.31 ± 1.061	32.48 ± 0.750
Enzyme Means	31.53 ± 0.484	31.47 ± 0.484	
	Fat Content (%)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	28.69 ± 2.930	30.59 ± 2.674	29.64 ± 1.983
10 % Canola Meal	29.10 ± 2.674	29.67 ± 2.674	29.38 ± 1.891
20 % Canola Meal	25.51 ± 2.674	25.00 ± 2.674	25.25 ± 1.891
10 % Juncea Meal	26.23 ± 2.674	27.12 ± 2.674	26.67 ± 1.891
20 % Juncea Meal	24.96 ± 2.674	22.35 ± 2.674	23.65 ± 1.891
Enzyme Means	26.89 ± 1.220	26.95 ± 1.196	

Table 6.7. Chi-square analysis for visually scored characteristics of livers from white-shell egg laying hens

	Hemorrhage Score		Colour Score	
	Enzyme	Meal	Enzyme	Meal
Chi-Square Statistic	1.70	12.79	0.12	1.68
Degrees of Freedom	4	16	1	4
P-value	0.791	0.688	0.729	0.794

ANOVA values for mortality due to FLHS (Table 6.8) indicated that the number of hens that died due to FLHS was not significant ($P > 0.05$). Of the 34 hens that died in the trial, only 6 of them had FLHS (17.6 %) which is equivalent to 1.7 % of the total flock. For mortality due to other causes, or total mortality, see Section 3.3.2. For a complete list of mortalities, see Appendix A, Table A.1.

Table 6.8. ANOVA *P*-values for mortality due to fatty liver hemorrhagic syndrome of white-shell egg laying hens

	Mortality due to Fatty Liver Hemorrhagic Syndrome
Source of Variation	
Enzyme	0.9999
Meal	0.2112
Period	0.8747
Enzyme*Meal	0.7745
Enzyme*Period	0.3715
Meal*Period	0.5545
Enzyme*Meal*Period	0.3936

6.6.2 Liver Measurements for Brown-Shell Egg Laying Hens

For Trial 2, enzyme supplementation decreased ‘b’ colour score (yellowness, Table 6.9) by 1.65 units (Table 6.10) but had no effect on ‘L’ or ‘a’ score ($P > 0.05$, Table 6.9). However, the ‘L’ and ‘a’ scores averaged 34.09 and 16.17, respectively (Table 6.10).

Table 6.9. ANOVA *P*-values for Hunter Lab colour scores for livers from brown-shell egg laying hens

	Score		
	L	a	b
Source of Variation			
Enzyme	0.0708	0.1217	0.0390
Meal	0.3087	0.3430	0.0614
Enzyme*Meal	0.9395	0.8495	0.6269

Table 6.10. Hunter Lab colour scores for livers from brown-shell egg laying hens

L Score			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	35.87 ± 1.315	34.68 ± 1.315	35.27 ± 0.930
10 % Canola Meal	35.54 ± 1.315	34.72 ± 1.315	35.13 ± 0.930
20 % Canola Meal	34.00 ± 1.315	32.13 ± 1.315	33.06 ± 0.930
10 % Juncea Meal	34.07 ± 1.315	33.09 ± 1.315	33.58 ± 0.930
20 % Juncea Meal	34.82 ± 1.315	32.00 ± 1.315	33.41 ± 0.930
Enzyme Means	34.86 ± 0.588	33.32 ± 0.588	
a Score			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	16.39 ± 0.601	17.20 ± 0.601	16.80 ± 0.425
10 % Canola Meal	15.91 ± 0.601	15.68 ± 0.601	15.79 ± 0.425
20 % Canola Meal	15.40 ± 0.601	15.99 ± 0.601	15.69 ± 0.425
10 % Juncea Meal	15.73 ± 0.601	16.50 ± 0.601	16.12 ± 0.425
20 % Juncea Meal	15.91 ± 0.601	16.96 ± 0.601	16.43 ± 0.425
Enzyme Means	15.87 ± 0.269	16.47 ± 0.269	
b Score			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	26.32 ± 1.234	23.74 ± 1.234	25.03 ± 0.873
10 % Canola Meal	23.46 ± 1.234	23.59 ± 1.234	23.53 ± 0.873
20 % Canola Meal	23.35 ± 1.234	19.85 ± 1.234	21.60 ± 0.873
10 % Juncea Meal	22.66 ± 1.234	21.41 ± 1.234	22.04 ± 0.873
20 % Juncea Meal	23.37 ± 1.234	22.29 ± 1.234	22.83 ± 0.873
Enzyme Means	**23.83[a] ± 0.552**	**22.18[b] ± 0.552**	

a-b enzyme means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Enzyme supplementation increased HSI (Table 6.11) by 0.09 units (Table 6.12). Meal type had a significant effect (Table 6.11) on % fat (Table 6.12). SBM had a higher liver fat content (27.82 %) than either 10 % JM (22.70 %) or 20 % CM (21.99 %). Percent DM followed the same general trend (*P* = 0.0850, Table 6.11) as % fat (Table 6.12), where the DM of livers from SBM fed hens was elevated more than the 20 % CM and the 10 % JM fed hens. There were no significant differences found for liver weight (Table 6.11). The measurements averaged 38.7 g (Table 6.12).

Table 6.11. ANOVA *P*-values for liver measurements of brown-shell egg laying hens

	Liver Weight	Hepatosomatic Index	Dry Matter	Fat Content
Source of Variation				
Enzyme	0.6287	0.0505	0.5765	0.6695
Meal	0.5523	0.4505	0.0850	0.0111
Enzyme*Meal	0.6212	0.5812	0.8689	0.9875

Chi-square analysis of hemorrhage score revealed that none of the livers received a score of zero (no hemorrhage) so this column was removed. The contingency tables were reduced from a 5x6 for meal and 5x2 for enzyme, to a 4x6 and a 4x2 respectively. The final results showed that there was no meal or enzyme effect on hemorrhage score ($P > 0.05$, Table 6.13).

The χ^2 analysis of meal effect on hemorrhage score could not be performed (Table 6.13) because only four of the livers received a score of 5 (rupture of Glisson's capsule). This caused expected counts of less than 1, indicating that the χ^2 statistic was not a good estimate for the distribution of hemorrhage score. Although conclusions could not be drawn for the effect of meal on hemorrhage score, the livers scored ranged from 1 (< 10 subcapsular petechial or ecchymotic hemorrhages) to 4 (hematomas with a diameter of > 2 cm), indicating that some liver damage was occurring in all hens, including the control (SBM) group.

Table 6.12. Liver measurements for brown-shell egg laying hens			
	Liver Weight (g)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	39.3 ± 1.68	42.1 ± 1.68	40.7 ± 1.19
10 % Canola Meal	39.6 ± 1.56	36.8 ± 1.68	38.2 ± 1.14
20 % Canola Meal	38.4 ± 1.84	38.5 ± 1.68	38.5 ± 1.25
10 % Juncea Meal	37.9 ± 1.68	39.1 ± 1.68	38.5 ± 1.19
20 % Juncea Meal	36.7 ± 1.68	38.4 ± 1.68	37.6 ± 1.19
Enzyme Means	38.4 ± 0.76	39.0 ± 0.75	
	Hepatosomatic Index (g liver / g body)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	1.83 ± 0.075	1.97 ± 0.075	1.90 ± 0.053
10 % Canola Meal	1.80 ± 0.075	1.76 ± 0.075	1.78 ± 0.053
20 % Canola Meal	1.81 ± 0.075	1.87 ± 0.075	1.84 ± 0.053
10 % Juncea Meal	1.77 ± 0.075	1.91 ± 0.075	1.84 ± 0.053
20 % Juncea Meal	1.69 ± 0.075	1.86 ± 0.075	1.78 ± 0.053
Enzyme Means	**1.78^{b} ± 0.034**	**1.87^{a} ± 0.034**	
	Dry Matter (%)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	31.73 ± 0.624	31.58 ± 0.624	31.65 ± 0.441
10 % Canola Meal	30.60 ± 0.624	30.27 ± 0.624	30.43 ± 0.441
20 % Canola Meal	30.51 ± 0.624	29.51 ± 0.624	30.01 ± 0.441
10 % Juncea Meal	30.09 ± 0.624	30.21 ± 0.624	30.15 ± 0.441
20 % Juncea Meal	30.54 ± 0.624	30.80 ± 0.624	30.67 ± 0.441
Enzyme Means	30.69 ± 0.279	30.47 ± 0.279	
	Fat Content (%)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	27.77 ± 1.731	27.86 ± 1.731	**27.82^{a} ± 1.224**
10 % Canola Meal	26.03 ± 1.602	25.25 ± 1.731	**25.64^{ab} ± 1.179**
20 % Canola Meal	21.75 ± 1.896	22.23 ± 1.731	**21.99^{b} ± 1.284**
10 % Juncea Meal	23.20 ± 1.731	22.20 ± 1.731	**22.70^{b} ± 1.224**
20 % Juncea Meal	26.00 ± 1.731	24.85 ± 1.731	**25.43^{ab} ± 1.224**
Enzyme Means	24.95 ± 0.779	24.48 ± 0.779	

a-b means ± SEM within main effects with different superscripts are significantly different $\alpha \leq 0.05$

Chi-square analysis of visual colour and texture scores (Table 6.13) found no meal or enzyme effect on these variables.

Table 6.13. Chi-square analysis for visually scored characteristics of livers from brown-shell egg laying hens

	Hemorrhage Score		Colour Score		Texture Score	
	Enzyme	Meal	Enzyme	Meal	Enzyme	Meal
Chi-Square Statistic	1.28	-	0.12	1.68	0.21	6.26
Degrees of Freedom	4	-	1	4	1	4
P-value	0.866	-	0.792	0.794	0.648	0.180

ANOVA values for mortality due to FLHS (Table 6.14) indicated that the number of hens that died due to FLHS in Trial 2 was not significant ($P > 0.05$). Of the 15 hens that died in the trial, only 4 of them had FLHS (26.7 %) which is equivalent to 1.3 % of the total flock. For mortality due to other causes, or total mortality, see Section 3.3.3. For a complete list of mortalities, see Appendix A, Table A.2.

Table 6.14. ANOVA *P*-values for mortality due to fatty liver hemorrhagic syndrome of brown-shell egg laying hens

	Mortality due to Fatty Liver Hemorrhagic Syndrome
Source of Variation	
Enzyme	0.3235
Meal	0.8998
Period	0.2409
Enzyme*Meal	0.4444
Enzyme*Period	0.9317
Meal*Period	0.6161
Enzyme*Meal*Period	0.2957

6.7 Discussion

6.7.1 Liver Measurements for White-Shell Egg Laying Hens

There were no previous reports with poultry that could verify or contradict the results for the Trial 1 Hunter Lab ‘L’, ‘a’ and ‘b’ colour score measurements. However, other studies have performed visual colour scoring as a method of determining FLHS in laying hens. Pearson and Butler (1978) found that injecting estrogen into immature birds resulted in enlarged, yellowish livers when chickens were fed *ad libitum*. Another study

found that laying hens receiving high energy corn-based diets also had enlarged, yellowish livers compared to control groups (Pearson et al. 1978a).

Another study focused on the effect of dietary cereal on hepatic lipid metabolism in hens. It found that feeding a corn-soy based diet to commercial Leghorns caused all hens to have friable livers with a large number of hemorrhages under the Glisson's capsule (Maurice and Jensen 1979).

Previous work done by Leeson et al. (1978) and Marangos and Hill (1976) supports the findings that there was no treatment effect on hemorrhage scores. In fact, both studies found liver hemorrhages in the control group as well as the group consuming the test diet.

Since diets containing CM or JM in the current study were corn-SBM based, this could explain why no differences were found in visual or Hunter Lab colour scores, or visual hemorrhage and texture scores of livers for WSLH. As well, all hens were sexually mature (thus producing estrogen), which caused almost all livers to appear yellowish-brown in colour.

Results for WSLH liver measurements (Table 6.6) agreed with past literature. Thomas et al. (1978) and Olomu et al. (1975) found that there was no change in liver weight with the inclusion of high and low glucosinolate RSM in the diet. Work by Kermanshahi and Abbasi Pour (2006), Smulikowska et al. (2006), and Cheva-Isarakul et al. (2001) agreed that there was no change in HSI when a multicarbohydrase enzyme, a phytase enzyme and RSM or mustard meal (respectively) were included in the diet. There was no treatment effect on DM or fat content which agreed with previous work using RSM (Olomu et al. 1975 and Thomas et al. 1978). Marangos and Hill (1976) established there was no difference in liver fat content when 12 % *Brassica napus* meal was included in the diet.

Previous work by Campbell (1979) found that hens consuming low-glucosinolate varieties of RSM did not have a significant increase in mortality, which concurred with the results of Trial 1. Further, the results of Trial 1 show the improvements that have been made to canola meal in the last 30 years, as Marangos et al. (1974) found high mortality rates attributed to FLHS when feeding 12 % of *Brassica napus* RSM or mustard seed.

6.7.2 Liver Measurements for Brown-Shell Egg Laying Hens.

As the same variables were measured in Trial 2 as in Trial 1, only those results that differ from Trial 1 will be discussed here. For an explanation of other results, see Section 6.7.1.

There were no previous studies done with poultry livers that could verify or contradict the results for the Trial 2 Hunter Lab 'b' colour score measurements. The 'b' score (positive 'b' is yellow) decreased from 23.83 to 22.18 with the addition of the enzyme cocktail (Table 6.10). 'L' colour score followed the same general trend as 'b' score, where the value of 'L' was slightly less with the addition of the enzyme cocktail (Table 6.10). While this decrease was not significant, the lower values did make biological sense as a liver that is less yellow (and therefore more blue), should also be less light (which was indicated by the reduction in 'L' score). One hypothesis for this decrease in colour was that with the addition of the enzyme, came a decrease in the amount of fat that was being deposited into the liver. However, Table 6.12 clearly indicated that there was no effect of enzyme on liver fat content. For future work, it may be a good idea to take liver colour measurements while the liver is still inside the body cavity to ensure that all measurements are taken from the same place on each liver. This may allow the colour measurement to more accurately reflect the condition of the liver, as there would be no tissue damage caused by removing the liver.

Enzyme supplementation increased HSI (Table 6.12) from 1.78 to 1.87. A study with laying hens found that 0.1 % of a NSP degrading enzyme included in the diet had no effect on HSI (Han et al. 2010), which may indicate that the change was due to the Bio-Phytase enzyme, not the Superzyme-OM^{TM}. This was supported by other studies where including 0.05 % of a multicarbohydrase in diets for broiler chickens had no effect on HSI (Kermanshahi and Abbasi Pour 2006 and Ahmadauli et al. 2008). A study done with rainbow trout (Lanari et al. 1998) found an increase in HSI when 1000 phytase U/ kg was added to a SBM based diet. This increase in HSI with the addition of phytase could be explained by a study done in 2010 with broilers (Karadas et al. 2010). Phytase supplementation of a phosphorus deficient diet resulted in an increase in liver α-tocopherol (vitamin E) and Coenzyme Q_{10} (Karadas et al. 2010). If the same thing happened in the livers of laying hens which are not phosphorus deficient, this could be

enough to slightly raise the weight of the liver, resulting in a higher HSI but not a significantly different weight. To determine if this is the cause, liver vitamin E and Coenzyme Q_{10} concentrations should be measured, and related to HSI and liver weight.

SBM-fed hens had a higher liver fat percentage (27.82 % fat) than 20 % CM or 10 % JM hens (21.99 % and 22.70 % fat, respectively). 'b' colour score (Table 6.10) and % DM (Table 6.12) followed the same general trend as % fat (Table 6.12). This was expected because livers with a higher fat content should be more yellow, and therefore have a higher 'b' score. Other studies evaluated the chemical composition of liver, and found that DM and fat content follow the same trend (Olomu et al. 1975, Maurice and Jensen 1979 and Caston et al. 1994).

Recent studies using CM or JM did not measure liver fat content of laying hens. However, research done by Leeson et al. (1976) found that corn-SBM control diet resulted in a lighter liver weight than the 5, 10 or 15 % RSM diets. These results contradicted the results of other research which found that there was no difference in liver fat content when RSM diets were fed (Olomu et al. 1975 and Marangos and Hill 1976).

Schumann et al. (2003) fed WSLH a flaxseed diet, a SBM-maize control diet, and each of these with a supplement designed to control the onset of FLHS. The resulting liver fat contents ranged from 22.0% to 27.4 % and no significant interactions were found. However diets containing flax were found to have significantly lower liver fat contents (22.0 % and 23.2 %) than diets without flax (26.9 % and 27.4 % liver fat). These results were similar to those found in this current study, as the SBM control diets had higher liver fat contents than some of the diets containing CM or JM.

Khajali and Slominski (2012) compared CM to SBM in a review of several research papers, and found that CM had a lower ME and higher fibre (lignin) content than SBM. As well, CM had a slightly lower carbohydrate content (Khajali and Slominski 2012) which hens use to synthesize fat, as most poultry diets do not contain a large amount of fats (Butler 1976). This could explain why 20 % CM had a lower liver fat content. If CM had a reduced amount of energy available to begin with, and reduced carbohydrates with which to synthesize fat, coupled with an increased rate of passage in the GI tract (due to higher fibre content) there would be a reduced energy intake and ultimately, less fat synthesized and stored in the liver.

While no research has been done to determine the normal range of liver fat for brown-shelled laying hens, these values closely matched normal ranges for white-shelled laying hens. Yousefi et al. (2005) fed WSLH a SBM control diet; the control with low methionine, low linoleic acid, high energy, or low choline; and the control with low methionine, low linoleic acid, low choline and high energy, for a total of 6 diets. There was no significant difference in liver fat content, and values ranged from 20.4 % to 27.6 % fat.

While it was determined that there was a difference between the SBM and the 20% CM and 10 % JM treatments for BSLH in this current study, the results are probably not biologically important because it was not the two CM treatments acting differently from the two JM treatments and the control, or the two 10 % treatments acting differently than the two 20 % treatments and the control. As well, all values obtained for liver fat percentage fall within ranges that were found in other studies (Schumann et al. 2003 and Yousefi et al. 2005).

6.7.3 Combined Trial Discussion

Results for Hunter Lab colour scores were very similar between the two trials for 'L' and 'a' score. The range in values was roughly the same, which indicated that the liver of each type of hen were the same colour in terms of lightness (L) and redness (a Table 6.15). BSLH had a wider range in values for 'b' score than WSLH (Table 6.15).

Liver measurements were similar between the two trials, although not as close as the colour measurements. WSLH had a wider range in values for liver weight, and had livers 3 g lighter than those of BSLH (Table 6.15). The white-shelled laying hens tended to have a higher HSI than the BSLH, which indicated that in terms of body size, the WSLH had a larger liver than the BSLH (Table 6.15). Similar results were found for DM and fat content of livers, where WSLH tended to have a higher DM and fat content percentage than BSLH (Table 6.15).

Table 6.15. Summary[1] of Hunter Lab colour scores and liver measurements for white- and brown-shell egg laying hens

	Range of Values	
	White Shelled Laying Hens	Brown Shelled Laying Hens
Hunter Lab Colour Score		
L	32.77 – 35.99	32.00 – 35.87
a	15.37 – 16.63	15.40 – 17.20
b	21.54 – 24.60	19.85 – 26.32
Liver Measurements		
Weight (g)	33.7 – 41.6	36.7 – 42.1
Hepatosomatic Index	1.87 – 2.32	1.69 – 1.97
Dry Matter (%)	30.17 – 33.40	29.51 – 31.73
Fat Content (%)	22.35 – 30.59	21.75 – 27.86

[1]Two trials were not compared statistically

While neither type of hen had significant treatment differences for liver colour, texture, or hemorrhage score, there were some difference between the hens. The first difference was that χ^2 statistic was not a good estimate for the distribution of texture score for the WSLH, but it was a good estimator for the BSLH. This indicated that although significant treatment differences were not detected, BSLH has a more variable texture score than WSLH.

Similarly, the χ^2 statistic was a good estimate of the distribution of hemorrhage score for white-shelled laying hens, but was not a good estimate of the distribution of hemorrhage score for meal, of the BSLH. This indicated that the meal hemorrhage score of the BSLH is less variable than the meal hemorrhage score of the WSLH.

Both WSLH and BSLH had similar mortality rates due to FLHS. For white-shelled laying hens FLHS accounted for 1.4 % of the total flock, while FLHS accounted for 1.3 % of the total flock for brown-shelled laying hens. Based on these two studies, FLHS previously associated with high glucosinolate rapeseed meal (Marangos et al. 1974) was not a factor in these hens.

6.8 Conclusions

There were no significant treatment effects on any of the liver characteristics measured including weight, hepatosomatic index, colour, texture, and hemorrhage score, dry matter, fat content, and mortality rate due to FLHS, when canola or juncea meal was included in the diet up to 20 % for white-shelled egg laying hens. These conclusions supported the hypothesis that canola or juncea meal can be used as a protein source and that enzyme supplementation would not have a significant effect on white-shelled egg laying hen liver health.

Feeding SBM to brown-shell egg laying hens resulted in a higher liver fat content than 20 % CM and 10 % JM, which was contrary to the hypothesis that there would be no significant effect of meal on liver characteristics. Since both inclusion levels of each meal were not affected by this trend, and the liver fat content was lower than the control, it can be concluded that up to 20 % CM or JM can be included in diets for brown-shell egg laying hens without negatively impacting the overall health of the hen.

Enzyme supplementation for brown-shell egg laying hens decreased 'b' colour score and increased HIS, but did not decrease liver fat content or increase liver weight. Therefore, the hypothesis that there would be no significant effect of enzyme on liver health of laying hens was rejected. However, since the changes were not found to be biologically important, enzyme can be included in the diets of brown-shell egg laying hens with no negative impact to liver health.

Based on the results it is recommended that up to 20 % CM or JM be fed to both white- and brown-shell egg laying hens. It is not necessary to include enzymes in the diet to improve liver health.

Chapter 7. Conclusions and Recommendations

Canola and juncea meals can be included at 20 % of the diet for both white- and brown-shell egg laying hens. Future work should evaluate the total replacement of protein supplied by soybean meal, with protein from canola or juncea meal, in diets for today's white- and brown-shell egg laying hens.

In some instances, 20 % juncea meal reduced body weight and feed consumption when compared to the soybean meal control. These results were not outside of normal ranges for the strains of hens used in this experiment and did not impact hen health or production performance. However, similar experiments should be conducted with other strains of hens to ensure that the same trends do not exist, or are not commercially important.

Enzyme supplementation improved production performance and bone quality measurements of white-shell egg laying hens, but did not significantly affect egg quality or liver health measurements. Enzymes improved production performance of brown-shell egg laying hens. Therefore, supplemental dietary enzymes should be included in diets for both white- and brown-shell egg laying hens.

Since the enzymes were used in combination in the diet, it was not always possible to determine which enzyme was having an effect on a measurement, or whether both were. For this reason, future work should evaluate both enzymes individually and in combination with each other. This way, it may be possible to determine which enzyme was effecting which measurement, and whether one or both of the enzymes are required in the diet for optimum performance and health.

The results of production performance, egg quality, bone quality, and liver health indicate that 20 % canola meal or 20 % juncea meal should be fed to both white- and brown-shell egg laying hens with the addition of dietary Biophytase and Superzyme-OM.

This study did not evaluate all factors that were known to be affected by consumption of RSM and CM in the past. The remaining factors include the effect of CM on thyroid function of commercial laying hens and on fishy egg taint in brown-shell egg laying hens. Since glucosinolates are known to effect thyroid function as well as liver health, this should be evaluated by comparing thyroid weight and blood plasma T3 concentrations in hens fed CM, JM and a control diet.

Fishy egg taint is caused by a genetic defect which does not allow hens to convert trimethylamine (TMA) to trimethylamine-oxide. TMA is produced when choline (bound in the form of sinapine) is fermented by gastrointestinal bacteria. Although breeder companies claim that the genetic defect has been removed from the hens through breeding, egg producers would like assurance that commercial hens are truly free from this costly problem. Therefore, future studies should use sensory methods to evaluate brown-shell eggs from hens fed CM and JM. Also, egg yolk TMA concentrations should be evaluated using an analytical method.

References

Ahmadauli, O., Eslami, M. and Fayazi, J. 2008. The effects of using the multi carbohydrase preparation in diets containing canola meal on performance of broiler chickens. Int. J. Poult. Sci. **7 (9):** 919-924.

Ahmed, A. M. H., Rodriguez, A., Vidal, M. L., Gautron, J., Garcia-Ruiz, J. M. and Nys, Y. 2003. Effect of moult on eggshell quality. Br. Poult. Sci. **44:** 782-783.

Akbar, M. K., Gavora, J. S., Friars, G. W. and Gowe, R. S. 1983. Composition of eggs by commercial size categories: effects of genetic group, age, and diet. Poult. Sci. **62:** 925-933.

Akiba, Y., Takahashi, K., Kimura, M., Hirama, S. and Matsumoto, T. 1983. The influence of environmental temperature, thyroid status and a synthetic oestrogen on the induction of fatty livers in chicks. Br. Poult. Sci. **24(1):** 71-80.

Al-Batshan, H. A., Scheideler, S. E., Black, B. L., Garlich, J. D. and Anderson, K. E. 1994. Duodenal calcium uptake, femur ash, and eggshell quality decline with and increase following molt. Poult. Sci. **73:** 1590-1596.

Alberta Egg Producers. 2010. All about eggs. [Online] Available: http://eggs.ab.ca/about-eggs/quality-grades [14 December 2010].

Association of Official Analytical Chemists. 2005. Official methods of analysis. 18th ed. AOAC International, Gaithersburg, MD.

Bain, M. M. 1997. A reinterpretation of eggshell strength. Pages 131-142 *in* S. E. Solomon, ed. Egg and Eggshell Quality. Manson Publishing Ltd., London, England.

Baker, R. C. and Vadehra. 1970. The influence of quantity of thick albumen on internal egg quality measurements. Poult. Sci. **49:** 493-496.

Bate-Smith, E. C. and Ribéreau-Gayon, P. 1959. Leuco-anthocyanins in seeds. Qual. Plant. Mater. Veg. **5(3):** 189-198.

Beare-Rogers, J. J. and Nera, E. A. 1972. Cardiac lipids in rats and gerbils fed oils containing C_{22} fatty acids. Lipids **7(8):** 548-552.

Bell, D. D. and Weaver, W. D., Jr. 2002. Commercial chicken meat and egg production. 5th ed. Springer Science + Business Media, Inc., New York, NY.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. **73:** 619-697.

Bell, J. M. and Shires, A. 1982. Composition and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats. Can. J. Anim. Sci. **62:** 557-565.

Bell, J. M., Keith, M. O. and Kowalenko, W. S. 1985. Digestibility and feeding value of frost-damaged canola (low glucosinolate rapeseed) for growing pigs. Can. J. Anim. Sci. **65(3):** 735-743.

Bell, J. M., Tyler, R. T. and Rakow, G. 1998. Nutritional composition and digestibility by 80-kg to 100-kg pigs of prepress solvent-extracted meals from low glucosinolate *Brassica juncea*, *B. napus* and *B. rapa* seed and of solvent-extracted soybean meal. Can. J. Anim. Sci. **78**: 199-203.

Bell, J. M., Keith, M. O., Blake, J. A. and McGregor, D. I. 1984. Nutritional evaluation of ammoniated mustard meal for use in swine feeds. Can. J. Anim. Sci. **64:** 1023-1033.

Bjerg, B., Eggum, B. O., Jacobsen, I., Otte, J. and Sørensen, H. 1989. Antinutritional and toxic effects in rats of individual glucosinolates (± myrosinases) added to a standard diet. J. Anim. Physiol. Anim. Nutr. **61:** 227-244.

Blakeslee, J. A. and Wilson, H. R. 1979. Response of hens to various dietary levels of tannic acid. Poult. Sci. **58(1):** 255 (Abstr.).

Boling, S. D., Douglas, M. W., Johnson, M. L., Wang, X., Parsons, C. M., Koelkebeck, K. W. and Zimmermen, R. A. 2000. The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult. Sci. **79:** 224-230.

Boorman, K. N. and Gunaratne, S. P. 2001. Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. Br. Poult. Sci. **42:** 81-91.

Bones, A. M. and Rossiter, J. T. 1996. The myrosinase-glucosinolate system, its organisation and biochemistry. Physiol. Plant. **97:** 194-208.

Bones, A. M., Thangstad, O. P., Haugen, O. A. and Espevik, T. 1991. Fate of myrosin cells: characterization of monoclonal antibodies against myrosinase. J. Exp. Bot. **42(245):** 1541-1549.

Brausch, J. M., Blackwell, B. R., Beall, B. N., Caudillo, C., Kolli, V., Godard-Codding, C., Cox, S. B., Cobb, G. and Smith, P. N. 2010. Effects of polycyclic aromatic hydrocarbons in northern bobwhite quail (Colinus virginianus). J. Toxicol. Environ. Health, Part A **73(8):** 540-551.

Burley, R. W. and Vadehra, D. V. 1989. The Avian Egg Chemistry and Biology. John Wiley and Sons, Inc. Toronto, Canada.

Butler, E. J. 1976. Fatty liver diseases in the domestic fowl – A review. Avian Pathol. **5(1):** 1-14.

Calderon, P., Van Buren, J. and Robinson, W. B. 1968. Factors influencing the formation of precipitates and hazes by gelatin and condensed and hydrolyzable tannins. J. Agric. Food Chem. **16(3):** 479-482.

Campbell, L. D. 1979. Incidence of liver haemorrhage among whiteleghorn strains fed on diets containing different types of rapeseed meals. Br. Poult. Sci. **20(3):** 239-246.

Canadian Council of Animal Care. 2009. CCAC guidelines on: the care and use of farm animals in research, teaching and testing. CCAC, Ottawa, ON.

Candlish. J. K. 1971. The formation of mineral and organic matrix of fowl cortical and medullary bone during shell calcification. Br. Poult. Sci. **12(1):** 119-127.

Canola Council of Canada. 2003. Canola growers manual: chapter 2 – canola varieties. [Online] Available: http://www.canola-council.org/chapter2.aspx [8 December 2010].

Canola Council of Canada. 2011. Poultry diets using canola meal. [Online] Available: http://www.canolacouncil.org/oil-and-meal/canola-meal/poultry-diets-using-canola-meal/ [3 January 2013].

Castillo, L., Tanaka, Y., Wineland, M. J., Jowsey, J. O. and Deluca, H. F. 1979. Production of 1,25-dihydroxyvitamin D_3 and formation of medullary bone in the egg-laying hen. Endocrinology **104(6):** 1598 (Abstr.).

Caston, L. J., Squires, E. J. and Leeson S. 1994. Hen performance, egg quality, and the sensory evaluation of eggs from SCWL hens fed dietary flax. Can. J. Anim. Sci. **74:** 347-353.

Chen, J. and Balnave, D. 2001. The influence of drinking water containing sodium chloride on performance and eggshell quality of a modern, colored layer strain. Poult. Sci. **80:** 91-94.

Cherian, G., Holsonbake, T. B., Goeger, M. P. and Bildfell, R. 2002. Dietary CLA alters yolk and tissue FA composition and hepatic histopathology of laying hens. Lipids **38(8):** 751-757.

Cheva-Isarakul, B., Tangtaweewipat, S. and Sangsrijun, P. 2001. The effect of mustard meal in laying hen diets. Asian-Aust. J. Anim. Sci. **14(11):** 1605-1609.

Choct, M. 2002. Non-starch polysaccharides: effect on nutritive value. Pages 221-235 *in* J. M. McNab and K. N. Boorman, eds. Poultry feedstuffs: supply, composition and nutritive value. CABI Publishing, Wallingford, UK.

Ciska, E., Martyniak-Przybyszewska, B. and Kozlowska, H. 2000. Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J. Agric. Food Chem. **48:** 2862-2867.

Cransberg, P. H., Parkinson, G. B., Wilson, S. and Thorp, B. H. 2001. Sequential studies of skeletal calcium reserves and structural bone volume in a commercial layer flock. Br. Poult. Sci. **42(2):** 260-265.

Czerwiński, J., Smulikowska, S., Mieczkowska, A., Konieczka, P., Piotrowska, A. and Bartkowiak-Broda, I. 2012. The nutritive value and phosphorus availability of yellow- and dark-seeded rapeseed cakes and the effects of phytase supplementation in broilers. J. Anim. Feed Sci. **21:** 677-695.

Dacke, C. G., Arkle, S., Cook, D. J., Wormstone, I. M., Jones, S., Zaidi, M. and Bascal, Z. M. 1993. Medullary bone and avian calcium regulation. J. Exp. Biol. **184:** 63-88.

de Bernard, B., Stagni, N., Camerotto, R., Vittur, F., Zanetti, M., Zambonin Zallone, A. and Teti, A. 1980. Influence of calcium depletion on medullary bone of laying hens. Calcif. Tissue Int. **32(1):** 221-228.

Diaz, G. J., Squires, E. J. and Julian, R. J. 1999. The use of selected plasma enzyme activities for the diagnosis of fatty liver-hemorrhagic syndrome in laying hens. Avian Dis. **43(4):** 768-773.

Dobush, G. R., Ankney, C. D. and Krementz, D. G. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. Can. J. Zool. **63:** 1917-1920.

Dorminey, R. W., Jones, J. E. and Wilson, H. R. 1965. Influence of cage size and frightening on incidence of body checked eggs. Poult. Sci. **44:** 307-308.

Duan, J. K. and McGregor, D. I. 1981. Glucosinolate analysis of rapeseed (canola). Method of the Canadian Grain Commission Grain Research Laboratory, Winnipeg, Manitoba.

Eapen, K. E., Tape, N. W. and Sims, R. P. A. 1968. New process for oil and meal. I. Effect of heat enzyme destruction and color the production of better-quality treatments on enzyme destruction and color of rapeseed oil. J. Am. Oil Chem. Soc. **45:** 194-196.

Ebrahimnezhad, Y., Shivazad, M., Taherkhani, R. and Nazeradl, K. 2008. Effects of citric acid and microbial phytase supplementation on performance and phytate phosphorus utilization in broiler chicks. J. Poult. Sci. **45:** 20-24.

Egg Farmers of Canada. 2013. Market Information – Producer Price List. [Online] Available: http://eggs.ca/industry-and-media/market-information [13 February 2013].

Egg Regulations. 2009. Canadian Agricultural Products Act. [Online] Available: http://laws-lois.justice.gc.ca [3 February 2011].

Eisen, E. J., Bohren, B. B. and McKean, H. E. 1962. The haugh unit as a measure of albumen quality. Poult. Sci. **41(5):** 1461-1468.

Etches, R. J. 1987. Calcium logistics in the laying hen. J. Nutr. **117:** 619-628.

Feeds Regulations. 1983. Feeds Act. [Online] Available: http://laws-lois.justice.gc.ca [25 January 2012].

Fernandez, X., Bouillier-Oudot , M., Molette , C., Bernadet , M. D. and Manse, H. 2011. Duration of transport and holding in lairage at constant postprandial delay to slaughter—Effects on fatty liver and breast muscle quality in mule ducks. Poult. Sci. **90:** 2360–2369.

Fernandez, X., Lahirigoyen, E., Bouillier-Oudot, M., Vitezica, Z. and Auvergne, A. 2010. The effects of stunning methods on product qualities in force-fed ducks and geese. 2. Fatty liver quality. Animal **4(1):** 139-146.

Fleming, R. H. 2008. Nutritional factors affecting poultry bone health. Proc. Nutr. Soc. **67:** 177-183.

Fleming, R. H., McCormack, H. A., McTeir, L. and Whitehead, C. C. 1996. Influence of medullary bone on humeral breaking strength. Br. Poult. Sci. **37**(Suppl. 1): S30-S32.

Fleming, R. H., Whitehead, C. C., Alvey, D., Gregory, N. G. and Wilkins, L. J. 1994. Bone structure and breaking strength in laying hens housed in different husbandry systems. Br. Poult. Sci. **35(5):** 651-662.

Fowler, N. G. 1990. Fatty liver syndrome in laying hens. Pages 316-317 *in* F. T. W. Jordan, ed. Poultry diseases. 3rd ed. Ballière Tindall, London, England.

Goldstein, J. L. and Swain, T. 1965. The inhibition of enzymes by tannins. Phytochemistry **4(1):** 185 (Abstr.).

Gordon, R. W. and Roland. D. A., Sr. 1997. Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. Poult. Sci. **76:** 1172-1177.

Gordon, R. W. and Roland, D. A., Sr. 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. Poult. Sci. **77:** 290-294.

Gough, A. W. and Weber, L. J. 1978. Massive liver hemorrhage in Ontario broiler chickens. Avian Dis. **22(1):** 205-210.

Green, T. J. and Innis, S. M. 2000. Low erucic acid canola oil does not induce heart triglyceride accumulation in neonatal pigs fed formula. Lipids **35(6):** 607-612.

Griffin, H. D., Guo, K., Windsor, D., and Butterwith, S. C. 1992. Adipose tissue lipogenesis and fat deposition in leaner broiler chickens. J. Nutr. **122:** 363-368.

Guillaume, J. and Bellec, R. 1977. Use of field beans (Vicia faba L) in diets for laying hens. Br. Poult. Sci. **18(5):** 573-583.

Gunasekera, C. P., Martin, L. D., Siddique, K. H. M. and Walton, G. H. 2006. Genotype by environment interactions of indian mustard (*Brassica juncea* L.) and canola (*B. napus* L.) in Mediterranean-type environments1. Crop growth and seed yield. Eur. J. Agron. **25(1):** 1-12.

Hamilton, R. M. G. 1982. Methods and factors that affect the measurement of eggshell quality. Poult. Sci. **61:** 2022-2039.

Han, Y. K., Jin, Y. H., Kim, J. H. and Thacker, P. A. 2010. Influence of enzyme and/or lysolecithin supplementation on performance, nutrient digestibility and egg quality for laying hens. Trends Anim. Vet. Sci. J. **1(1):** 28-35.

Harms, R. H., Simpson, C. F. and Damron, B. L. 1972. Some new observations on "fatty liver syndrome" in laying hens. Avian Dis. **16(5):** 1042-1046.

Hermier, D., Catheline, D. and Legrand, P. 1996. Relationship between hepatic fatty acid desaturation and lipid secretion in the estrogenized chicken. Comp. Biochem. Physiol. **155A(3):** 259-264.

Hester, P. Y., Wilson, D. A., Settar, P., Arango, J. A. and O'Sullivan, N. P. 2011. Effect of lighting programs during the pullet phase on skeletal integrity of egg-laying strains of chickens. Poult. Sci. **90:** 1645-1651.

Hocking, P. M., Bain, M., Channing, C. E., Fleming, R. and Wilson, S. 2003. Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. Br. Poult. Sci. **44(3):** 65-373.

Höglund, A., Lenman, M., Falk, A. and Rask, L. 1991. Distribution of myrosinase in rapeseed tissues. Plant Physiol. **95:** 213-221.

Holtrop, M. E., Raisz, L. G. and Simmons, H. A. 1974. The effects of parathyroid hormone, colchicine, and calcitonin on the ultrastructure and the activity of osteoclasts in organ culture. J. Cell Biol. **60:** 346-355.

Hudson, H. A., Britton, W. M., Rowland, G. N. and Buhr, R. J. 1993. Histomorphometric bone properties of sexually immature and mature white leghorn hens with evaluation of fluorochrome injection on egg production traits. Poult. Sci. **72:** 1537-1547.

Hulan, H. W. and Proudfoot, F. G. 1980. The nutritional value of rapeseed meal for caged layers. Can. J. Anim. Sci. **60:** 139-147.

HunterLab. 2008a. Insight on colour: Colourimeters versus spectrophotometers. Application Notes. [Online] Available: http://www.hunterlab.com/appnotes/an03_95r.pdf [21 November 2012].

HunterLab. 2008b. Insight on colour: Hunter L, a, b colour scale. Application Notes. [Online] Available: http://www.hunterlab.com/appnotes/an08_96a.pdf [20 August 2012].

HunterLab. 2008c. Insight on colour: Hunter L, a, b versus CIE 1976 L*a*b*. Application Notes. [Online] Available: http://www.hunterlab.com/appnotes/an02_01.pdf [27 March 2013].

Ibrahim, I. K. and Hill, R. 1980. The effects of rapeseed meal from *Brassica napus* varieties and the variety Tower on the production and health of laying fowl. Br. Poult. Sci. **21(6):** 423-430.

Jalal, M. A. and Scheideler, S. E. 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci. **80:** 1463-1471.

Jendral, M. J., Korver, D. R., Church, J. S. and Feddes, J. J. R. 2008. Bone mineral density and breaking strength of white leghorns housed in conventional, modified, and commercially available colony battery cages. Poult. Sci. **87:** 828-837.

Jia, W., Slominski, B. A., Guenter, W., Humphreys, A. and Jones, O. 2008. The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed. Poult. Sci. **87:** 2005-2014.

Joyner, C. J., Peddie, M. J. and Taylor, T. G. 1987. The effect of age on egg production in the domestic hen. Gen. Comp. Endocrinol. **65(3):** 331(Abstr.).

Kallio, D. M., Garant, P. R. and Minkin, C. 1972. Ultrastructural effects of calcitonin on osteoclasts in tissue culture. J. Ultrastruct. Res. **39:** 205-216.

Karadas, F., Pirgozliev, V., Pappas, A. C., Acamovic, T. and Bedford, M. R. 2010. Effects of different phytase activities on the concentration of antioxidants in the liver of growing broilers. J. Anim. Physiol. Anim. Nutr. **94:** 519-526.

Kermanshahi, H. and Abbasi Pour, A. R. 2006. Replacement value of soybean meal with rapeseed meal supplemented with or without a dietary NSP-degrading enzyme on performance, carcass traits and thyroid hormones of broiler chickens. Int. J. Poult. Sci. **5(10):** 932-937.

Khajali, F. and Slominski, B. A. 2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. **91:** 2564-2575.

Kim, W. K., Bloomfield, S. A., Sugiyama, T. and Ricke, S. C. 2012. Concepts and methods for understanding bone metabolism in laying hens. World's Poult. Sci. J. **68:** 71-82.

Korver, D. R., Saunders-Blades, J. L. and Nadeau, K. L. 2004. Assessing bone mineral density in vivo: Quantitative computed tomography. Poult. Sci. **83:** 223-229.

Koutoulis, K. C., Kyriazakis, I., Perry, G. C. and Lewis, P. D. 2009. Effect of different calcium sources and calcium intake on shell quality and bone characteristics of laying hens at sexual maturity and end of lay. Int. J. Poult. Sci. **8(4):** 342-348.

Kovacs-Nolan, J., Phillips, M. and Mine, Y. 2005. Advances in the value of eggs and egg components for human health. J. Agric. Food Chem. **53:** 8421-8431.

Krumbein, A., Schonhof, I. and Schreiner, M. 2005. Composition and contents of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected Brassica species (B. juncea, B. rapa subsp. nipposinica var. chinoleifera, B. rapa subsp. chinensis and B. rapa subsp. rapa). J. Appl. Bot. Food Qual. **79(3):** 168 (Abstr.).

Lanari, D., D'Agaro, E. and Turri, C. 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture **161:** 345-356.

Lázaro, R., Garcia, M., Aranibar, M. J. and Mateos, G. G. 2003. Effect of enzyme addition to wheat-, barley- and rye-based diets on nutrient digestibility and performance of laying hens. Br. Poult. Sci. **44(2):** 256-265.

Lee, K-H., Olomu, J. M. and Sim, J. S. 1991. Live performance, carcass yield, protein and energy retentionof broiler chickens fed canola and flax full-fat seeds and there stored mixtures of meal and oil. Can. J. Anim. Sci. **71:** 897-903.

Leeson, S., Atteh, J. O. and Summers, J. D. 1987a. Effects of increasing dietary levels of full-fat canola on performance, nutrient retention, and bone mineralization. Poult. Sci. **66(5):** 875 (Abstr.).

Leeson, S., Atteh, J. O. and Summers, J. D. 1987b. The replacement value of canola meal for soybean meal in poultry diets. Can. J. Anim. Sci. **67:** 151-158.

Leeson, S., Caston, L. and Namkung, H. 2007. Effect of dietary lutein and flax on performance, egg composition and liver status of laying hens. Can. J. Anim. Sci. **87**: 365-372.

Leeson, S., Slinger, S. J. and Summers, J. D. 1978. Utilization of whole tower rapeseed by laying hens and broiler chickens. Can. J. Anim. Sci. **58:** 55-61.

Leeson, S., Summers, J. D. and Grandhi, R. 1976. Carcass characteristics and performance of laying birds fed rapeseed meal. Can. J. Anim. Sci. **56:** 551-558.

Leske, K. L. and Coon, C. N. 1999. A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. Poult. Sci. **78:** 1151-1157.

Leslie, A. J. and Summers, J. D. 1972. Feeding value of rapeseed for laying hens. Can. J. Anim. Sci. **52:** 562-566.

Leung, J., Fenton, T. W., Mueller, M. M. and Clandinin, D. R. 1979. Condensed tannins of rapeseed meal. J. Food Sci. **44:** 1313-1316.

Leveille, G. A., Romsos, D. R., Yeh, Y. and O'Hea, E. K. 1975. Lipid biosynthesis in the chick. A consideration of site of synthesis, influence of diet and possible regulatory mechanisms. Poult. Sci. **54:** 1075-1093.

Littell, R. C., Milliken, G. A., Stroup, W. W. and Wolfinger, R. D. 1996. SAS system for mixed models.SAS Institute, Cary, NC.

Lohmann Tierzucht. 2007. Lohmann LSL-Lite. North American Edition. Cuxhaven, Germany.

Lohmann Tierzucht. 2010. Lohmann Pedigree - Layers. [Online] Available: http://www.ltz.de/produkte/Layers/ [1 February 2011].

Lokaewmanee, K., Yamauchi, K., Komori, T. and Saito K. 2011. Enhancement of yolk color in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. J. Poult. Sci. **48:** 25-32.

Love, H. K., Rakow, G., Raney, J. P. and Downey, R. K. 1990. Development of low glucosinolate mustard. Can. J. Plant Sci. **70:** 419-424.

Mackie, E. J., Ahmed, Y. A., Tatarczuch, L., Chen, K. and Mirams, M. 2008. Endochondral ossification: how cartilage is converted to bone in the developing skeleton. Int. J. Biochem. Cell Biol. **40:** 46-62.

Mailer, R. J. and Cornish, P. S. 1987. Effects of water stress on glucosinolate and oil concentrations in the seeds of rape (*Brassica napus* L.) and turnip rape (*Brassica rapa* L. var. *slivestris* [Lam.] Briggs). Aust. J. Exp. Agric. **27:** 707-711.

Marangos, A. G. and Hill, R. 1976. The use of rapeseed meals and a mustard seed meal as a protein source in diets for laying pullets. Br. Poult. Sci. **17(6):** 643-653.

Marangos, A., Hill, R., Laws, B. M. and Muschamp, D. 1974. The influence of three rapeseed meals and a mustard seed meal on egg and broiler production. Br. Poult. Sci. **15(4):** 405-414.

March, B. E., Biely, J. and Soong, R. 1972. Rapeseed meal in the chicken breeder diet. Effects on production, mortality, hatchability and progeny. Poult. Sci. **51:** 1589-1596.

Marion, W. W., Nordskog, A. W., Tolman, H. S. and Forsythe, R. H. 1964. Egg composition as influenced by breeding, egg size, age and season. Poult. Sci. **43:** 255-264.

Martland, M. F., Butler, E. J. and Fenwick, G. R. 1984. Rapeseed induced liver haemorrhage, reticulolysis and biochemical changes in laying hens: the effects of feeding high and low glusosinolate meals. Res. Vet. Sci. **36(3):** 298-309.

Maurice, D. V. and Jensen, L. S. 1979. Hepatic lipid metabolism in domestic fowl as influenced by dietary cereal. J. Nutr. **109:** 872-882.

McCutcheon, J. S., Umermura, T., Bhatnagar, M. K. and Walker, B. L. 1976. Cardiopathogenicity of rapeseed oils and oil blends differing in erucic, linoleic, and linolenic acid content. Lipids **11(7):** 545-552.

McKinnon, P. J. and Bowland, J. P. 1979. Effects of feeding low and high glucosinolate rapeseed meals and soybean meal on thyroid function of young pigs. Can. J. Anim. Sci. **59:** 589-596.

McVetty, P. B. E. and Scarth, R. 2002. Breeding for improved oil quality in brassica oilseed species. J.Crop Prod. **5(1-2):** 345-369.

Meng, X., Slominski, B. A., Nyachoti, C. M., Campbell, L. D. and Guenter, W. 2005. Degradation of cell wall polysaccharides by combinationsof carbohydrase enzymes and their effect on nutrient utilizationand broiler chicken performance. Poult. Sci. **84(1):** 37-47.

Mithen, R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica **63:** 71-83.

Moore, D. S., McCabe, G. P. and Craig, B. A. 2009. Introduction to the practice of statistics. 6th ed. W.H. Freeman and Company, New York, NY. 525 pp.

Morra, M. J. and Kirkegaard, J. A. 2002. Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biol. Biochem. **34(11):** 1683-1690.

Naczk, M., Nichols, T., Pink, D. and Sosulski, F. 1994. Condensed tannins in canola hulls. J. Agric. Food Chem. **42:** 2196-2200.

Najib, H. and Al-Khateeb, S.A. 2004. The effect of incorporating different levels of locally produced canola seeds (*Brassica napus*, L.) in the diet of laying hen. Int. J. Poult. Sci. **3(7):** 490-496.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry 9th Revised Edition. National Academy of Sciences. Washington, D.C.

Newkirk, R. (ed). 2009. Canola Meal Feed Industry Guide. Canola Council of Canada. 4th ed. Winnipeg, MB.

Newkirk, R. W., Classen, H. L. and Edney, M. J. 2003a. Effects of pre-pressed solvent extraction on the nutritional value of canola meal for broiler chickens. Anim. Feed Sci. Tech. **104:** 111-119.

Newkirk, R.W., Classen, H. L. and Tyler, R.T. 1997. Nutritional evaluation of low glucosinolate mustard meals (*Brassica juncea*) in broiler diets. Poult. Sci. **76:** 1272-1277.

Newkirk, R. W., Classen, H. L., Scott, T. A. and Edney, M. J. 2003b. The availability and content of amino acids in toasted and non-toasted canola meals. Can. J. Anim. Sci. **83**: 131–139.

Nørgaard-Nielsen, G. 1990. Bone strength of laying hens kept in an alternative system, compared with hens in cages and on deep-litter. Br. Poult. Sci. **31(1):** 81-89.

Northcutt, J. K., Savage, S. I. and Vest, L. R. 1997. Relationship between feed withdrawal and viscera condition of broilers. Poult. Sci. **76:** 410-414.

Oh, H. I., Hoff, J. E., Armstrong, G. S. and Haff, L. A. 1980. Hydrophobic interaction in tannin-protein complexes. J. Agric. Food Chem. **28(2):** 394-398.

Olomu, J. M., Robblee, A. R., Clandinin, D. R. and Hardin, R. T. 1975. Effects of Span rapeseed meal on productive performance, egg quality, composition of liver and hearts and incidence of "fatty livers" in laying hens. Can. J. Anim. Sci. **55:** 7l-75.

Pallauf, J. and Rimbach, G. 1997. Nutritional significance of phytic acid and phytase. Arch. Anim. Nutr. **50(4):** 301-319.

Pearson, A. W. and Butler, E. J. 1978. The oestrogenised chick as an experimental model for fatty liver-haemorrhagic syndrome in the fowl. Res. Vet. Sci. **24:** 82-86.

Pearson, A. W., Arkhipov, A. V., Butler, E. J. and Laursen-Jones, A. P. 1978a. Influence of dietary cereal and energy content on the accumulation of lipids in the liver in fatty liver-haemorrhagic syndrome in the fowl. Res. Vet. Sci. **24:** 72-76.

Pearson, A. W., Butler, E. J., Curtis, R. F., Fenwick, G. R., Hobson-Frohock, A., Land, D. G. and Hall, S. A. 1978b. Effects of rapeseed meal on laying hens (Gallus domesticus) in relation to fatty liver-haemorrhagic syndrome and egg taint. Res. Vet. Sci. **25(3):** 307 (Abstr.).

Potts, D. A., Rakow, G. W. and Males, D. R. 1999. Canola-quality *brassica juncea*, a new oilseed crop for the Canadian prairies. [Online] Available: http://regional.org.au/au/gcirc/4/70.htm. [30 June 2010].

Rath, N. C., Huff, G. R., Huff, W. E. and Balog, J. M. 2000. Factors regulating bone maturity and strength. Poult. Sci. **79:** 1024-1032.

Ravindran, V., Cabahug, S., Ravindran, G. and Bryen, W. L. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. **78(5):** 699-706.

Rennie, J. S., Fleming, R. H., McCormack, H. A., McCorquodale, C. C. and Whitehead, C. C. 1997. Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. Br. Poult. Sci. **38(4):** 417-424.

Riczu, C. M., Saunders-Blades, J. L., Yngvesson, Å. K., Robinson, F. E. and Korver, D. R. 2004. End-of-cycle bone quality in white- and brown-egg laying hens. Poult. Sci. **83:** 375-383.

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

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

Roland, D. A., Sr., Sloan, D. R. and Harms, R. H. 1975. The ability of hens to maintain calcium deposition in the egg shell and egg yolk as the hen ages. Poult. Sci. **54:** 1720-1723.

Rouvinen-Watt, K., Mustonen, A.-M., Conway, R., Pal, C., Harris, L., Saarela, S., Strandberg, U. and Nieminen, P. 2010. Rapid development of fasting-induced hepatic lipidosis in the american mink (Neovison vison): Effects of food deprivation and re-alimentation on body fat depots, tissue fatty acid profiles, hematology and endocrinology. Lipids **45:** 111-128.

Rowghani, E., Arab, M., Nazifi, S. and Bakhtiari, Z. 2007. Effect of canola oil on cholesterol and fatty acid composition of egg-yolk of laying hens. Int. J. Poult. Sci. **6(2):** 111-114.

Sang, J. P., Minchinton, I. R., Johnstone, P. K. and Truscott, R. J. W. 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Can. J. Plant Sci. **64:** 77-93.

Saunders-Blades, J. L., MacIssac, J. L., Korver, D. R. and Anderson, D. M. 2009. The effect of calcium source and particle size on the production performance and bone quality of laying hens. Poult. Sci. **88:** 338-353.

Schöne, F., Rudolph, B., Kirchheim, U. and Knapp, G. 1997. Counteracting the negative effects of rapeseed and rapeseed press cake in pig diets. Br. J. Nutr. **78:** 947-962.

Schumann, B. E., Squires, E. J., Leeson, S. and Hunter, B. 2003. Effect of hens fed dietary flaxseed with and without a fatty liver supplement on hepatic, plasma and production characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. Br. Poult. Sci. **44(2):** 234-244.

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

Silversides, F. G. and Hruby, M. 2009. Feed formulation using phytase in laying hen diets. J. Appl. Poult. Res. **18:** 15-22.

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

Silversides, F. G., Korver, D. R and Budgell, K. L. 2006a. Effect of strain of layer and age at photostimulation on egg production, egg quality, and bone strength. Poult. Sci. **85:** 1136-1144.

Silversides, F. G., Singh, R., Cheng, K. M. and Korver, D. R. 2012. Comparison of bones of 4 strains of laying hens kept in conventional cages and floor pens. Poult. Sci. **91:** 1-7.

Silversides, F. G., Scott, T. A., Korver, D. R., Afsharmanesh, M. and Hruby, M. 2006b. A study on the interaction of xylanase and phytase enzymes in wheat-based diets fed to commercial white and brown egg laying hens. Poult. Sci. **85:** 297-305.

Simbaya, J., Slominski, B. A., Guenter, W., Morgan, A. and Campbell, L. D. 1996. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: In vitro and in vivo studies. Anim. Feed Sci. Tech. **61:** 219-234.

Simbaya, J., Slominski, B. A., Rakow, G., Campbell, L. D., Downey, R. K. and Bell, J. M. 1995. Quality Characteristics of Yellow-seeded *Brassica* seed meals: Protein, carbohydrates, and dietary fiber components. J. Agric. Food Chem. **43:** 2062-2066.

Sjaastad, Ø. V., Hove, K. and Sand, O. 2003. Physiology of domestic animals. Scandinavian Veterinary Press, Oslo.

Slominski, B. A., Simbaya, J., Campbell, L. D., Rakow, G. and Guenter, W. 1999. Nutritive value for broilers of meals derived from newly developed varieties of yellow-seeded canola. Anim. Feed Sci. Tech. **78:** 249-262.

Smith, T. K. and Campbell, L. D. 1976. Rapeseed meal glucosinolates: metabolism and effect on performance in laying hens. Poult. Sci. **55(3):** 861 (Abstr.).

Smulikowska, S., Mieczkowska, A., Czerwiński, J., Weremko, D. and Nguyen, C. V. 2006. Effects of exogenous phytase in chickens fed diets with differently processed rapeseed expeller cakes. J. Anim. Feed Sci. **15:** 237-252.

Solomon, S. E. 1997. Egg and Eggshell Quality. Manson Publishing Ltd., London, England. Pg 11-33.

Stadelman, W. J. 1995. Quality identification of shell eggs. Pages 39-66 *in* W. J. Stadelman and O. J. Cotterill, eds. Egg science and technology. The Haworth Press, Inc., Binghamton, NY, USA.

Stefansson, B. R. and Kondra, Z. P. 1975. Tower summer rape. Can. J. Plant Sci. **55(1):** 343-344.

Stowe, K. A. 1998. Realized defence of artificially selected lines of *Brassica rapa*: Effects of quantitative genetic variation in foliar glucosinolate concentration. Environ. Entomol. **27(5):** 1166-1174.

Summers, J. D. and Leeson, S. 1985. Trace mineral profile of canola and soybean meal. Can. J. Anim. Sci. **65:** 913-919.

Summers, J. D., Hunt, E. C. and Leeson, S. 1985. Canola meal for laying hens. Can. J. Anim. Sci. **65:** 905-911.

Summers, J. D., Leeson, S. and Spratt, D. 1987. Canola meal and egg size. Can. J. Anim. Sci. **60:** 907-913.

Sun, J. M., Richards, M. P., Rosebrough, R. W., Ashwell, C. M., McMurtry, J. P. and Coon, C. N. 2006. The relationship on body composition, feed intake, and metabolic hormones for broiler breeder females. Poult. Sci. **85:** 1173-1184.

Talebali, H. and Farzinpour, A. 2005. Effect of different levels of full-fat canola seed as a replacement for soybean meal on the performance of broiler chickens. Int. J. Poult. Sci. **4(12):** 982-985.

Tangtaweewipat, S., Cheva-Isarakul, B. and Sangsrijun, P. 2004. The use of mustard meal as a protein source in broiler diets. Songklanakarin J. Sci. Technol. **26(1):** 23-30.

Taylor, T. G. and Belanger, L. F. 1969. The mechanism of bone resorption in laying hens. Calcif. Tissue Int. **4(1):** 162-173.

Thomas, D., Robblee, A. R. and Clandinin, D. R. 1978. Effects of low and high glucosinolate rapeseed meals on productive performance, egg quality, composition of liver and incidence of haemorrhagic liver syndrome in laying birds. Br. Poult. Sci. **19(4):** 449-454.

Tona, K., Bamelis, F., De Ketelaere, B., Bruggeman, V. and Decuypere, E. 2002. Effect of induced molting on albumen quality, hatchability, and chick body weight from broiler breeders. Poult. Sci. **81:** 327-332.

Trampel, D. W., Sell, J. L., Ahn, D. U. and Sebranek, J. G. 2005. Preharvest feed withdrawal affects liver lipid and liver color in broiler chickens. Poult. Sci. **84:** 137-142.

Tripathi, M. K. and Mishra, A. S. 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Tech. **132(1):** 1-27.

Turcsán, Zs., Szigeti, J., Varga, L., Farkas, L., Birkás, E. and Turcsán, J. 2001. The effects of electrical and controlled atmosphere stunning methods on meat and liver quality of geese. Poult. Sci. **80:** 1647–1651.

Turner, C. H. 2002. Biomechanics of bone: Determinants of skeletal fragility and bone quality. Osteoporosis Int. **13:** 97-104.

Um, J. S. and Paik, I. K. 1999. Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci. **78:** 75-79.

Vandepopuliere, J. M. and Lyons, J. J. 1992. Effect of inorganic phosphate source and dietary phosphorus level on laying hen performance and eggshell quality. Poult. Sci. **71:** 1022-1031.

van der Klis, J. D., Versteegh, H. A. J., Simons, P. C. M. and Kies, A. K. 1997. The efficacy of phytase in corn-soybean meal-based diets for laying hens. Poult. Sci. **76:** 1535-1542.

Velleman, S. G. 2000. The role of the extracellular matrix in skeletal development. Poult. Sci. **79:** 985-989.

Vogtmann, H., Clandinin, D. R. and Hardin, R. T. 1974. The influence of high and low erucic acid rapeseed oils on the productive performance of laying hens and on the lipid fraction of egg yolk. Can. J. Anim. Sci. **54:** 403-410.

Wells, R. G. 1968. The measurement of certain egg quality characteristics: a review. Pages 207-250 *in* T. C. Carter, ed. Egg quality: a study of the hen's egg. Oliver and Boyd, Edinburgh.

Whitehead, C. C. 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. **83:** 193-199.

Whitehead, C. C. and Wilson, S. 1992. Characteristics of osteopenia in hens. Pages 265-280 *in* C. C. Whitehead, ed. Bone biology and skeletal disorders in poultry. Carfax Publishing Company, Oxfordshire, England.

Wolford, J. H. and Polin, D. 1972. Lipid accumulation and hemorrhage in livers of laying chickens. A study on fatty liver-hemorrhagic Syndrome (FLHS). Poult. Sci. **51:** 1707-1713.

Yang, Y-H., Wang, Y., Lu, Y. and Li, Q-Z. 2011. Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion on rainbow trout (Oncorhynchus mykiss). Aquacult. Int. **19:** 405–419.

Yousefi, M., Shivazad, M. and Sohrabi-Haghdoost, I. 2005. Effect of dietary factors on induction of fatty liver-hemorrhagic syndrome and its diagnosis methods with use of serum and liver parameters in laying hens. Int. J. Poult. Sci. **4(8):** 568-572.

Zhang, B. and Coon, C. N. 1997. The relationship of various tibia bone measurements in hens. Poult. Sci. **76:** 1698-1701.

Appendix A. Nutrient composition of canola and juncea.

Table A.1 Nutrient composition of canola (*B. napus*) and juncea (*B. juncea*)

Component (%)	*B. napus*	*B. juncea*
Moisture	11.80[1]	10.79
Crude Protein[2]	43.80	43.92
Amino Acids		
Taurine	0.09	0.09
Hydroxyproline	0.36	0.26
Aspartic Acid	3.03	3.31
Threonine	1.83	1.80
Serine	1.71	1.63
Glutamic Acid	6.86	6.88
Proline	2.48	2.30
Lanthionine	0.02	0.02
Glycine	2.12	2.20
Alanine	1.89	1.97
Cysteine	1.04	0.93
Valine	1.94	2.23
Methionine	0.86	0.83
Isoleucine	1.46	1.76
Leucine	2.94	3.15
Tyrosine	1.17	1.20
Phenylalanine	1.64	1.74
Hydroxylysine	0.15	0.06
Ornithine	0.01	0.01
Lysine	2.27	2.25
Histidine	1.09	1.12
Arginine	2.54	2.84
Tryptophan	0.49	0.46
Crude Fat	2.17	1.93
Crude Fiber	9.64	8.25
Ash	8.79	8.23
Acid Detergent Fibre	20.44	15.08
Neutral Detergent Fibre	30.60	22.27
Starch	0.00	1.86
Calcium	0.75	0.80
Phosphorus	1.43	1.57

[1]Results on dry matter basis except moisture
[2]Percentage N x 6.25

Appendix B. Three-way interaction tables for production performance measurements for white-shell egg laying hens.

Table B.1. Three-way interaction means ± standard errors for feed consumption (g/hen/day) of white-shell egg laying hens

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	112 ± 1.9	112 ± 2.0	111 ± 1.9	112 ± 1.9	106 ± 1.9	111 ± 0.6
2	114 ± 1.6	113 ± 1.6	112 ± 1.6	110 ± 1.6	106 ± 1.6	111 ± 0.6
3	114 ± 2.1	112 ± 2.1	113 ± 2.1	109 ± 2.1	106 ± 2.1	110 ± 0.7
4	114 ± 2.2	112 ± 2.2	111 ± 2.2	109 ± 2.2	104 ± 2.2	110 ± 0.7
5	113 ± 2.4	113 ± 2.4	111 ± 2.4	109 ± 2.4	109 ± 2.4	111 ± 0.8
6	115 ± 3.0	115 ± 3.0	111 ± 3.0	110 ± 3.0	111 ± 3.0	111 ± 0.9
7	114 ± 3.1	114 ± 3.1	113 ± 3.1	111 ± 3.1	110 ± 3.1	110 ± 1.0
8	114 ± 4.0	109 ± 4.0	117 ± 4.0	114 ± 4.0	107 ± 4.0	110 ± 1.3
9	114 ± 3.9	115 ± 3.9	116 ± 3.9	113 ± 3.9	111 ± 3.9	112 ± 1.2
10	114 ± 4.3	114 ± 4.3	113 ± 4.3	110 ± 4.3	108 ± 4.3	111 ± 1.4
11	113 ± 4.9	118 ± 4.9	112 ± 4.9	114 ± 4.9	111 ± 4.9	114 ± 1.6
12	117 ± 4.3	115 ± 4.3	112 ± 4.3	112 ± 4.3	112 ± 4.3	114 ± 1.4
Yes						
1	114 ± 1.9	109 ± 1.9	114 ± 1.9	110 ± 1.9	111 ± 1.9	
2	112 ± 1.6	110 ± 1.6	109 ± 1.7	112 ± 1.6	110 ± 1.7	
3	113 ± 2.1	110 ± 2.1	110 ± 2.1	110 ± 2.1	107 ± 2.1	
4	113 ± 2.2	110 ± 2.2	108 ± 2.2	108 ± 2.2	108 ± 2.2	
5	116 ± 2.4	109 ± 2.4	110 ± 2.4	108 ± 2.4	107 ± 2.4	
6	111 ± 3.0	109 ± 3.0	111 ± 3.0	108 ± 3.0	109 ± 3.0	
7	108 ± 3.1	109 ± 3.1	110 ± 3.1	107 ± 3.1	108 ± 3.1	
8	111 ± 4.0	106 ± 4.0	112 ± 4.0	106 ± 4.0	108 ± 4.0	
9	112 ± 3.9	111 ± 3.9	113 ± 3.9	107 ± 3.9	112 ± 3.9	
10	113 ± 4.3	110 ± 4.3	114 ± 4.3	106 ± 4.3	110 ± 4.3	
11	115 ± 4.9	113 ± 4.9	119 ± 4.9	112 ± 4.9	114 ± 4.9	
12	115 ± 4.3	113 ± 4.3	120 ± 4.3	111 ± 4.3	114 ± 4.3	
Meal $\bar{x}$	113 ± 1.7	112 ± 1.7	113 ± 1.7	110 ± 1.7	109 ± 1.7	
Enzyme $\bar{x}$						
No			112 ± 1.1			
Yes			111 ± 1.1			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table B.2. Three-way interaction means ± standard errors for body weight (g) of white-shell egg laying hens

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	1623 ± 23.6	1636 ± 23.6	1634 ± 23.6	1592 ± 23.6	1564 ± 23.6	1611 ± 7.5
1	1651 ± 23.8	1654 ± 23.8	1664 ± 23.8	1629 ± 23.8	1586 ± 23.8	1646 ± 7.5
2	1709 ± 26.7	1706 ± 26.7	1700 ± 26.7	1650 ± 26.7	1597 ± 26.7	1678 ± 8.5
3	1703 ± 28.7	1719 ± 28.7	1717 ± 28.7	1673 ± 28.7	1612 ± 28.7	1692 ± 9.1
4	1755 ± 32.2	1745 ± 32.2	1732 ± 32.2	1694 ± 32.2	1626 ± 32.2	1716 ± 10.2
5	1787 ± 31.7	1784 ± 31.7	1751 ± 31.7	1712 ± 31.7	1632 ± 31.7	1744 ± 10.0
6	1818 ± 34.6	1815 ± 34.6	1768 ± 34.6	1736 ± 34.6	1664 ± 34.6	1764 ± 10.9
7	1862 ± 42.8	1791 ± 42.8	1779 ± 42.8	1758 ± 42.8	1673 ± 42.8	1774 ± 13.5
8	1850 ± 36.4	1825 ± 36.4	1804 ± 36.4	1790 ± 36.4	1693 ± 36.4	1788 ± 11.5
9	1888 ± 43.6	1834 ± 43.6	1836 ± 43.6	1794 ± 43.6	1729 ± 43.6	1804 ± 13.8
10	1823 ± 42.2	1815 ± 42.2	1862 ± 42.2	1766 ± 42.2	1677 ± 42.2	1773 ± 13.3
11	1766 ± 39.6	1801 ± 39.6	1759 ± 39.6	1729 ± 39.6	1654 ± 39.6	1748 ±12.5
12	1759 ± 37.7	1804 ± 37.7	1764 ± 37.7	1701 ± 37.7	1664 ± 37.7	1751 ±11.9
Yes						
0	1640 ± 23.6	1566 ± 23.6	1636 ± 23.6	1614 ± 23.6	1611 ± 23.6	
1	1675 ± 23.8	1598 ± 23.8	1683 ± 23.8	1663 ± 23.8	1655 ± 23.8	
2	1745 ± 26.7	1617 ± 26.7	1707 ± 26.7	1675 ± 26.7	1679 ± 26.7	
3	1739 ± 28.7	1634 ± 28.7	1733 ± 28.7	1711 ± 28.7	1677 ± 28.7	
4	1785 ± 32.2	1661 ± 32.2	1733 ± 32.2	1743 ± 32.2	1689 ± 32.2	
5	1847 ± 31.7	1709 ± 31.7	1747 ± 31.7	1766 ± 31.7	1701 ± 31.7	
6	1876 ± 34.6	1704 ± 34.6	1768 ± 34.6	1777 ± 34.6	1720 ± 34.6	
7	1874 ± 42.8	1752 ± 42.8	1758 ± 42.8	1785 ± 43.3	1709 ± 42.8	
8	1890 ± 36.4	1724 ± 36.4	1773 ± 36.4	1789 ± 36.4	1742 ± 36.4	
9	1898 ± 43.6	1750 ± 43.6	1756 ± 43.6	1804 ± 43.6	1748 ± 43.6	
10	1872 ± 42.2	1702 ± 42.2	1752 ± 42.2	1760 ± 42.2	1705 ± 42.2	
11	1877 ± 39.6	1690 ± 39.6	1759 ± 39.6	1755 ± 39.6	1693 ± 39.6	
12	1881 ± 37.7	1711 ± 37.7	1763 ± 37.7	1750 ± 37.7	1715 ± 37.7	
Meal $\bar{x}$	1792 ± 21.6	1721 ± 21.6	1744 ± 21.6	1724 ± 21.6	1670 ± 21.6	
Enzyme $\bar{x}$						
No			1727 ± 13.7			
Yes			1733 ± 13.7			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table B.3. Three-way interaction means ± standard errors for hen-day egg production (%) of white-shell eggs

Enzyme Period	Meal SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	96.6 ± 1.03	97.3 ± 1.03	97.6 ± 1.03	99.3 ± 1.03	96.5 ± 1.03	97.0 ± 0.32
2	97.7 ± 1.11	98.1 ± 1.11	96.4 ± 1.11	97.9 ± 1.11	96.0 ± 1.11	96.7 ± 0.35
3	97.0 ± 2.05	94.2 ± 2.05	96.0 ± 2.05	97.3 ± 2.05	94.2 ± 2.05	95.0 ± 0.65
4	96.4 ± 1.31	96.5 ± 1.31	97.4 ± 1.31	96.2 ± 1.31	93.4 ± 1.31	96.2 ± 0.42
5	96.9 ± 0.98	96.6 ± 0.98	94.9 ± 0.98	93.9 ± 0.98	95.0 ± 0.98	95.5 ± 0.31
6	92.5 ± 1.64	91.8 ± 1.64	88.5 ± 1.64	92.6 ± 1.64	91.6 ± 1.64	92.3 ± 0.52
7	95.8 ± 1.78	95.2 ± 1.78	93.6 ± 1.78	93.0 ± 1.78	93.0 ± 1.78	93.8 ± 0.56
8	93.7 ± 1.70	93.0 ± 1.70	94.9 ± 1.70	91.3 ± 1.70	94.8 ± 1.70	93.5 ± 0.54
9	92.2 ± 1.76	91.6 ± 1.76	92.8 ± 1.76	91.1 ± 1.76	90.9 ± 1.76	91.6 ± 0.56
10	89.9 ± 2.10	90.4 ± 2.10	86.5 ± 2.10	83.9 ± 2.10	85.6 ± 2.10	87.5 ± 0.66
11	90.7 ± 2.40	90.9 ± 2.40	86.4 ± 2.40	84.5 ± 2.40	89.9 ± 2.56	89.0 ± 0.77
12	85.5 ± 2.33	86.0 ± 2.33	80.8 ± 2.33	77.5 ± 2.33	80.7 ± 2.33	83.9 ± 0.74
Yes						
1	96.0 ± 1.03	97.2 ± 1.03	98.5 ± 1.03	93.8 ± 1.03	96.9 ± 1.03	
2	93.0 ± 1.11	97.8 ± 1.11	97.2 ± 1.11	97.3 ± 1.11	95.7 ± 1.18	
3	91.6 ± 2.05	96.6 ± 2.05	97.5 ± 2.05	91.8 ± 2.05	94.2 ± 2.05	
4	96.6 ± 1.39	97.9 ± 1.31	97.2 ± 1.31	93.7 ± 1.31	96.3 ± 1.31	
5	96.3 ± 0.98	96.2 ± 0.98	95.5 ± 0.98	94.8 ± 1.05	94.9 ± 0.98	
6	93.8 ± 1.64	95.4 ± 1.64	94.2 ± 1.64	89.0 ± 1.64	93.9 ± 1.64	
7	93.5 ± 1.78	93.4 ± 1.78	95.4 ± 1.78	91.2 ± 1.78	93.6 ± 1.78	
8	93.1 ± 1.81	94.0 ± 1.70	95.2 ± 1.70	90.0 ± 1.70	94.5 ± 1.70	
9	90.1 ± 1.76	91.3 ± 1.76	94.0 ± 1.76	88.0 ± 1.76	93.9 ± 1.76	
10	88.9 ± 2.10	88.6 ± 2.10	90.0 ± 2.10	82.2 ± 2.10	89.5 ± 2.10	
11	91.3 ± 2.40	90.8 ± 2.40	89.6 ± 2.40	84.8 ± 2.40	91.4 ± 2.40	
12	85.1 ± 2.33	83.9 ± 2.33	89.4 ± 2.33	81.4 ± 2.33	89.1 ± 2.33	
Meal $\bar{x}$	93.1 ± 0.72	93.5 ± 0.72	93.3 ± 0.72	90.7 ± 0.72	92.7 ± 0.73	
Enzyme $\bar{x}$						
No			92.5 ± 0.46			
Yes			92.8 ± 0.46			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table B.4. ANOVA *P*-values for hen-housed egg production of white-shell egg laying hens

Source of Variation	Hen-Housed Egg Production
Enzyme	0.9589
Meal	0.7374
Period	< 0.0001
Enzyme*Meal	0.3327
Enzyme*Period	0.4179
Meal*Period	0.4755
Enzyme*Meal*Period	0.3485

Table B.5. Three-way interaction means ± standard errors for hen-housed egg production (%) of white-shell egg laying hens

Enzyme	Meal					
Period	SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	96.6 ± 2.15	97.3 ± 2.15	92.2 ± 2.15	99.3 ± 2.15	96.5 ± 2.15	96.5 [a] ± 0.68
2	98.3 ± 3.65	95.3 ± 3.65	88.5 ± 3.65	98.0 ± 3.65	96.0 ± 3.65	95.1 [a] ± 1.15
3	97.0 ± 4.28	94.2 ± 4.28	88.2 ± 4.28	97.3 ± 4.28	88.9 ± 4.28	93.0 [b] ± 1.35
4	96.4 ± 4.15	93.8 ± 4.15	89.1 ± 4.15	96.2 ± 4.15	85.2 ± 4.15	92.0 [b] ± 1.31
5	95.0 ± 3.98	93.9 ± 3.98	86.8 ± 3.98	93.9 ± 3.98	84.4 ± 3.98	90.6 [b] ± 1.26
6	89.3 ± 3.89	89.2 ± 3.89	81.2 ± 3.89	92.6 ± 3.89	81.3 ± 3.89	87.5 [cd] ± 1.23
7	90.4 ± 4.54	92.7 ± 4.54	86.2 ± 4.54	91.3 ± 4.54	82.3 ± 4.54	88.0 [c] ± 1.44
8	88.4 ± 4.40	90.5 ± 4.40	87.1 ± 4.40	86.5 ± 4.40	84.2 ± 4.40	86.7 [cd] ± 1.39
9	86.8 ± 4.34	89.1 ± 4.34	84.9 ± 4.34	86.1 ± 4.34	79.5 ± 4.34	84.9 [d] ± 1.37
10	84.6 ± 4.36	87.8 ± 4.36	79.7 ± 4.36	78.1 ± 4.36	76.0 ± 4.36	80.6 [e] ± 1.38
11	85.3 ± 5.13	87.3 ± 5.13	79.4 ± 5.13	76.3 ± 5.13	75.0 ± 5.13	80.8 [e] ± 1.62
12	80.7 ± 4.93	81.1 ± 4.93	74.3 ± 4.93	69.7 ± 4.93	69.1 ± 4.93	76.1 [f] ± 1.56
Yes						
1	96.2 ± 2.15	97.2 ± 2.15	98.5 ± 2.15	93.8 ± 2.15	97.0 ± 2.15	
2	90.0 ± 3.65	97.8 ± 3.65	97.3 ± 3.65	95.1 ± 3.65	94.2 ± 3.65	
3	88.9 ± 4.28	95.8 ± 4.28	97.5 ± 4.28	91.8 ± 4.28	90.5 ± 4.28	
4	88.3 ± 4.15	94.3 ± 4.15	97.2 ± 4.15	91.2 ± 4.15	88.4 ± 4.15	
5	88.3 ± 3.98	90.9 ± 3.98	95.5 ± 3.98	90.5 ± 3.98	86.9 ± 3.98	
6	85.8 ± 3.89	90.0 ± 3.89	94.2 ± 3.89	86.4 ± 3.89	84.9 ± 3.89	
7	85.6 ± 4.54	85.5 ± 4.54	94.0 ± 4.54	88.7 ± 4.54	83.4 ± 4.54	
8	85.6 ± 4.45	83.4 ± 4.40	92.5 ± 4.40	84.8 ± 4.40	83.7 ± 4.40	
9	82.7 ± 4.34	81.2 ± 4.34	91.7 ± 4.34	83.2 ± 4.34	83.4 ± 4.34	
10	78.7 ± 4.36	78.6 ± 4.36	85.5 ± 4.36	77.7 ± 4.36	79.6 ± 4.36	
11	78.6 ± 5.13	80.5 ± 5.13	84.6 ± 5.13	80.3 ± 5.13	81.4 ± 5.13	
12	71.1 ± 4.93	74.0 ± 4.93	84.5 ± 4.93	77.0 ± 4.93	79.2 ± 4.93	
Meal $\bar{x}$	87.9 ± 2.55	89.2 ± 2.55	88.8 ± 2.55	87.7 ± 2.55	84.6 ± 2.55	
Enzyme $\bar{x}$						
No			87.7 ± 1.61			
Yes			87.6 ± 1.61			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

a-f period means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Table B.6. Three-way interaction means ± standard errors for feed conversion ratio (kg feed/kg egg mass) of white-shell egg laying hens

Enzyme	Meal					
Period	SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	1.98 ± 0.066	1.93 ± 0.066	1.88 ± 0.066	1.89 ± 0.066	1.88 ± 0.066	1.94 ± 0.021
2	1.89 ± 0.066	1.92 ± 0.066	1.97 ± 0.066	1.87 ± 0.066	1.90 ± 0.066	1.92 ± 0.021
3	1.99 ± 0.066	1.98 ± 0.066	1.99 ± 0.066	1.88 ± 0.066	1.97 ± 0.066	1.97 ± 0.021
4	1.90 ± 0.066	1.91 ± 0.066	1.90 ± 0.066	1.86 ± 0.066	1.91 ± 0.066	1.91 ± 0.021
5	1.90 ± 0.066	1.92 ± 0.066	1.95 ± 0.066	1.93 ± 0.066	1.95 ± 0.066	1.92 ± 0.021
6	1.99 ± 0.066	2.04 ± 0.070	2.08 ± 0.070	1.90 ± 0.066	2.04 ± 0.066	1.97 ± 0.021
7	1.89 ± 0.066	1.96 ± 0.066	1.95 ± 0.066	1.92 ± 0.066	1.97 ± 0.066	1.92 ± 0.021
8	1.90 ± 0.066	1.92 ± 0.066	2.00 ± 0.066	1.98 ± 0.066	1.87 ± 0.066	1.92 ± 0.021
9	2.00 ± 0.066	2.00 ± 0.066	2.03 ± 0.066	1.97 ± 0.066	1.97 ± 0.066	1.97 ± 0.021
10	1.99 ± 0.066	1.99 ± 0.066	2.09 ± 0.066	2.06 ± 0.066	1.99 ± 0.066	2.04 ± 0.021
11	2.00 ± 0.066	2.06 ± 0.066	2.02 ± 0.066	2.16 ± 0.066	2.02 ± 0.070	2.04 ± 0.021
12	2.25 ± 0.070	2.06 ± 0.066	2.16 ± 0.066	2.24 ± 0.070	2.23 ± 0.066	2.15 ± 0.021
Yes						
1	2.03 ± 0.066	1.90 ± 0.066	1.94 ± 0.066	2.04 ± 0.066	1.95 ± 0.066	
2	2.01 ± 0.066	1.93 ± 0.066	1.87 ± 0.070	1.96 ± 0.066	1.93 ± 0.070	
3	2.02 ± 0.066	1.98 ± 0.066	1.92 ± 0.066	2.00 ± 0.066	1.95 ± 0.066	
4	2.00 ± 0.066	1.96 ± 0.066	1.84 ± 0.066	1.93 ± 0.066	1.90 ± 0.066	
5	1.99 ± 0.066	1.86 ± 0.066	1.91 ± 0.066	1.92 ± 0.066	1.86 ± 0.066	
6	1.97 ± 0.066	1.88 ± 0.066	1.92 ± 0.066	1.98 ± 0.066	1.89 ± 0.066	
7	1.90 ± 0.066	1.88 ± 0.066	1.93 ± 0.070	1.92 ± 0.066	1.95 ± 0.066	
8	1.91 ± 0.070	1.86 ± 0.066	1.97 ± 0.066	1.91 ± 0.066	1.87 ± 0.066	
9	1.97 ± 0.066	1.97 ± 0.066	1.96 ± 0.066	1.90 ± 0.066	1.95 ± 0.066	
10	2.07 ± 0.066	2.06 ± 0.066	2.06 ± 0.066	2.04 ± 0.066	2.01 ± 0.066	
11	1.98 ± 0.066	1.98 ± 0.066	2.08 ± 0.066	2.09 ± 0.066	2.01 ± 0.066	
12	2.14 ± 0.066	2.14 ± 0.066	2.10 ± 0.070	2.12 ± 0.066	2.07 ± 0.066	
Meal $\bar{x}$	1.99 ± 0.032	1.96 ± 0.032	1.98 ± 0.032	1.98 ± 0.032	1.96 ± 0.032	
Enzyme $\bar{x}$						
No			1.98 ± 0.020			
Yes			1.97 ± 0.020			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Appendix C. Three-way interaction tables for production performance measurements for brown-shell egg laying hens.

Table C.1. Three-way interaction means ± standard errors for feed consumption (g/hen/day) of brown-shell egg laying hens

Enzyme Period	Meal SBM	 10 % CM	 20 % CM	 10 % JM	 20 % JM	Period $\bar{x}$
No						
1	118 ± 2.2	116 ± 2.2	114 ± 2.2	115 ± 2.2	115 ± 2.2	115 ± 0.7
2	120 ± 2.0	119 ± 2.0	114 ± 2.0	116 ± 2.0	113 ± 2.0	115 ± 0.6
3	116 ± 2.6	115 ± 2.6	109 ± 2.6	111 ± 2.6	108 ± 2.6	111 ± 0.8
4	115 ± 2.5	115 ± 2.5	111 ± 2.5	117 ± 2.5	112 ± 2.5	112 ± 0.8
5	114 ± 2.1	110 ± 2.1	108 ± 2.1	113 ± 2.1	109 ± 2.1	109 ± 0.7
6	114 ± 2.6	114 ± 2.6	111 ± 2.6	113 ± 2.6	112 ± 2.6	111 ± 0.8
7	112 ± 2.3	110 ± 2.3	111 ± 2.3	112 ± 2.3	110 ± 2.3	109 ± 0.7
8	115 ± 2.5	111 ± 2.5	112 ± 2.5	110 ± 2.5	113 ± 2.5	111 ± 0.8
9	110 ± 2.7	111 ± 2.7	112 ± 2.7	109 ± 2.7	110 ± 2.7	110 ± 0.9
10	108 ± 2.7	109 ± 2.7	110 ± 2.7	105 ± 2.7	107 ± 2.7	108 ± 0.9
11	111 ± 3.0	109 ± 3.0	113 ± 3.0	107 ± 3.0	109 ± 3.0	111 ± 0.9
12	114 ± 3.4	114 ± 3.4	115 ± 3.4	109 ± 3.4	110 ± 3.4	113 ± 1.1
Yes						
1	114 ± 2.2	112 ± 2.2	117 ± 2.2	115 ± 2.2	110 ± 2.2	
2	113 ± 2.0	113 ± 2.0	116 ± 2.0	117 ± 2.0	110 ± 2.0	
3	110 ± 2.6	114 ± 2.6	110 ± 2.6	112 ± 2.6	104 ± 2.6	
4	111 ± 2.5	109 ± 2.5	113 ± 2.5	112 ± 2.5	106 ± 2.5	
5	109 ± 2.1	106 ± 2.1	112 ± 2.1	109 ± 2.1	105 ± 2.1	
6	110 ± 2.6	109 ± 2.6	113 ± 2.6	108 ± 2.6	107 ± 2.6	
7	107 ± 2.3	105 ± 2.3	108 ± 2.3	110 ± 2.3	105 ± 2.3	
8	107 ± 2.5	107 ± 2.5	114 ± 2.5	112 ± 2.5	108 ± 2.5	
9	108 ± 2.7	106 ± 2.7	113 ± 2.7	113 ± 2.7	109 ± 2.7	
10	111 ± 2.7	105 ± 2.7	116 ± 2.7	108 ± 2.7	105 ± 2.7	
11	112 ± 3.0	108 ± 3.0	118 ± 3.0	115 ± 3.0	113 ± 3.0	
12	114 ± 3.4	108 ± 3.4	120 ± 3.5	118 ± 3.4	113 ± 3.4	
Meal $\bar{x}$	112 ± 1.4	111 ± 1.4	113 ± 1.4	112 ± 1.4	109 ± 1.4	
Enzyme $\bar{x}$						
No			112 ± 0.9			
Yes			111 ± 0.9			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table C.2. Three-way interaction means ± standard errors for body weight (g) of brown-shell egg laying hens

Enzyme Period	Meal SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	1983 ± 36.4	2033 ± 36.4	1949 ± 36.4	2027 ± 36.4	2030 ± 36.4	1991 ± 11.5
1	2041 ± 46.0	2102 ± 46.0	2004 ± 46.0	2076 ± 46.0	2087 ± 46.0	2056 ± 14.5
2	2109 ± 38.6	2176 ± 38.6	2041 ± 38.6	2118 ± 38.6	2118 ± 38.6	2092 ± 12.2
3	2112 ± 38.0	2179 ± 38.0	2035 ± 38.0	2101 ± 38.0	2106 ± 38.0	2093 ± 12.0
4	2165 ± 46.7	2216 ± 46.7	2036 ± 46.7	2153 ± 46.7	2116 ± 46.7	2108 ± 14.8
5	2260 ± 60.5	2321 ± 59.6	2076 ± 59.6	2168 ± 59.6	2197 ± 59.6	2169 ± 18.9
6	2185 ± 46.6	2277 ± 46.6	2078 ± 46.6	2179 ± 46.6	2171 ± 46.6	2149 ± 14.7
7	2204 ± 54.2	2278 ± 53.4	2058 ± 53.4	2174 ± 53.4	2176 ± 53.4	2149 ± 16.9
8	2242 ± 57.0	2334 ± 57.0	2101 ± 57.0	2243 ± 57.0	2224 ± 57.0	2181 ± 18.0
9	2225 ± 55.3	2274 ± 55.3	2107 ± 55.3	2211 ± 55.3	2192 ± 55.3	2164 ± 17.5
10	2158 ± 46.5	2246 ± 46.5	2061 ± 46.5	2179 ± 46.5	2168 ± 46.5	2125 ± 14.7
11	2167 ± 46.1	2234 ± 46.1	2066 ± 46.1	2174 ± 46.1	2165 ± 46.1	2123 ± 14.6
12	2159 ± 46.2	2257 ± 46.2	2076 ± 46.2	2146 ± 46.2	2145 ± 46.2	2126 ± 14.6
Yes						
0	1949 ± 36.4	1971 ± 36.4	1990 ± 36.4	2003 ± 36.4	1975 ± 36.4	
1	2009 ± 46.0	2025 ± 46.0	2054 ± 46.0	2123 ± 46.0	2041 ± 46.0	
2	2049 ± 38.6	2078 ± 38.6	2090 ± 38.6	2092 ± 38.6	2047 ± 38.6	
3	2051 ± 38.0	2075 ± 38.0	2091 ± 38.0	2100 ± 38.0	2077 ± 38.0	
4	2074 ± 46.7	2086 ± 46.7	2054 ± 46.7	2107 ± 46.7	2074 ± 46.7	
5	2159 ± 59.6	2149 ± 59.6	2112 ± 59.6	2137 ± 59.6	2112 ± 59.6	
6	2127 ± 46.6	2127 ± 46.6	2091 ± 46.6	2139 ± 46.6	2116 ± 46.6	
7	2097 ± 53.4	2154 ± 53.4	2107 ± 53.4	2124 ± 53.4	2115 ± 53.4	
8	2148 ± 57.0	2157 ± 57.0	2084 ± 57.0	2172 ± 57.0	2110 ± 57.0	
9	2106 ± 55.3	2160 ± 55.3	2070 ± 55.3	2149 ± 55.3	2151 ± 55.3	
10	2095 ± 46.5	2082 ± 46.5	2069 ± 46.5	2129 ± 46.5	2069 ± 46.5	
11	2082 ± 46.1	2058 ± 46.1	2068 ± 46.1	2116 ± 46.1	2100 ± 46.1	
12	2127 ± 46.2	2079 ± 46.2	2099 ± 46.2	2095 ± 46.2	2076 ± 46.2	
Meal $\bar{x}$	2119 ± 30.5	2159 ± 30.5	2064 ± 30.5	2132 ± 30.5	2114 ± 30.5	
Enzyme $\bar{x}$						
No			2146 ± 19.2			
Yes			2089 ± 19.2			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table C.3. Three-way interaction means ± standard errors for hen-day egg production (%) of brown-shell egg laying hens

Enzyme	Meal					
Period	SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	96.7 ± 1.76	97.7 ± 1.76	97.5 ± 1.76	95.4 ± 1.76	97.7 ± 1.76	96.5 ± 0.56
2	96.0 ± 1.95	97.0 ± 1.95	96.7 ± 1.95	97.1 ± 1.95	97.1 ± 1.95	95.9 ± 0.62
3	95.2 ± 1.61	94.9 ± 1.61	96.1 ± 1.61	96.0 ± 1.61	95.3 ± 1.61	95.4 ± 0.51
4	92.1 ± 2.00	91.8 ± 2.00	94.8 ± 2.00	95.5 ± 2.00	94.5 ± 2.00	93.5 ± 0.63
5	86.3 ± 2.45	88.3 ± 2.45	92.1 ± 2.45	92.7 ± 2.45	92.4 ± 2.45	90.7 ± 0.77
6	88.9 ± 2.50	86.2 ± 2.50	89.5 ± 2.50	89.5 ± 2.50	90.5 ± 2.50	88.9 ± 0.79
7	90.5 ± 2.43	85.2 ± 2.43	88.2 ± 2.43	89.9 ± 2.43	90.9 ± 2.43	88.5 ± 0.77
8	89.7 ± 2.62	86.1 ± 2.62	91.3 ± 2.62	89.6 ± 2.62	91.9 ± 2.63	89.3 ± 0.83
9	85.1 ± 2.84	86.0 ± 2.84	89.5 ± 2.84	88.1 ± 2.84	90.5 ± 2.84	87.5 ± 0.90
10	82.6 ± 2.95	81.4 ± 2.95	85.1 ± 2.95	82.2 ± 2.95	85.2 ± 2.95	82.9 ± 0.93
11	81.1 ± 3.36	86.5 ± 3.36	85.1 ± 3.36	84.2 ± 3.36	87.7 ± 3.36	84.9 ± 1.07
12	80.0 ± 3.44	85.4 ± 3.44	81.4 ± 3.44	79.5 ± 3.44	84.5 ± 3.58	81.4 ± 1.10
Yes						
1	96.6 ± 1.76	97.0 ± 1.76	96.1 ± 1.76	96.5 ± 1.76	93.9 ± 1.76	
2	93.7 ± 1.95	94.9 ± 1.95	96.0 ± 1.95	97.5 ± 1.95	93.0 ± 1.95	
3	96.4 ± 1.61	95.4 ± 1.61	96.8 ± 1.61	96.2 ± 1.61	91.8 ± 1.61	
4	94.0 ± 2.00	93.5 ± 2.00	94.3 ± 2.00	95.0 ± 2.00	89.4 ± 2.00	
5	90.6 ± 2.45	91.4 ± 2.45	93.2 ± 2.45	91.9 ± 2.45	87.5 ± 2.45	
6	90.0 ± 2.50	89.2 ± 2.50	91.4 ± 2.50	88.5 ± 2.50	84.8 ± 2.50	
7	87.6 ± 2.43	88.6 ± 2.43	88.7 ± 2.43	90.6 ± 2.43	84.5 ± 2.43	
8	88.9 ± 2.62	87.6 ± 2.62	89.2 ± 2.62	91.4 ± 2.62	87.7 ± 2.62	
9	87.3 ± 2.84	83.7 ± 2.84	86.9 ± 2.84	90.6 ± 2.84	87.6 ± 2.84	
10	79.6 ± 2.95	77.9 ± 2.95	87.2 ± 2.95	89.4 ± 2.95	78.5 ± 2.95	
11	84.1 ± 3.49	79.6 ± 3.36	90.1 ± 3.36	87.7 ± 3.36	83.1 ± 3.36	
12	82.6 ± 3.61	75.4 ± 3.44	85.7 ± 3.44	83.1 ± 3.44	76.6 ± 3.44	
Meal $\bar{x}$	89.0 ± 0.36	88.4 ± 0.36	91.0 ± 0.36	90.8 ± 0.36	89.0 ± 0.36	
Enzyme $\bar{x}$						
No			89.9 ± 0.86			
Yes			89.3 ± 0.86			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table C.4. ANOVA *P*-values for hen-housed egg production of brown-shell egg laying hens

	Hen-Housed Egg Production
Source of Variation	
Enzyme	0.3242
Meal	0.9813
Period	< 0.0001
Enzyme*Meal	0.5954
Enzyme*Period	0.9455
Meal*Period	0.4201
Enzyme*Meal*Period	0.8323

Table C.5. Three-way interaction means ± standard errors for body weight (g) of brown-shell egg laying hens

Enzyme	Meal					
Period	SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	96.7 ± 2.10	97.7 ± 2.10	97.5 ± 2.10	95.4 ± 2.10	98.0 ± 2.10	96.5 [a] ± 0.66
2	96.0 ± 2.54	97.0 ± 2.54	96.7 ± 2.54	97.1 ± 2.54	94.4 ± 2.54	95.5[ab] ± 0.80
3	95.2 ± 2.01	94.9 ± 2.01	96.0 ± 2.01	96.0 ± 2.01	92.0 ± 2.01	94.7 [b] ± 0.63
4	92.1 ± 2.53	91.8 ± 2.53	94.8 ± 2.53	92.6 ± 2.53	91.2 ± 2.53	92.2 [c] ± 0.80
5	86.3 ± 3.03	88.3 ± 3.03	92.1 ± 3.03	89.4 ± 3.03	89.2 ± 3.03	89.2 [d] ± 0.96
6	87.5 ± 3.15	86.2 ± 3.15	89.5 ± 3.15	86.5 ± 3.15	87.3 ± 3.15	86.9 [e] ± 1.00
7	87.3 ± 2.82	85.2 ± 2.82	88.2 ± 2.82	86.8 ± 2.82	87.7 ± 2.82	86.1 [e] ± 0.89
8	86.5 ± 3.33	86.0 ± 3.33	91.3 ± 3.33	86.7 ± 3.33	88.8 ± 3.33	86.8 [e] ± 1.05
9	81.9 ± 3.59	86.0 ± 3.59	89.5 ± 3.59	85.5 ± 3.59	86.6 ± 3.59	84.8 [e] ± 1.14
10	79.6 ± 3.97	81.4 ± 3.97	85.1 ± 3.97	78.9 ± 3.97	82.8 ± 3.97	80.6 [f] ± 1.26
11	78.0 ± 5.24	84.9 ± 5.24	85.1 ± 5.24	79.2 ± 5.24	82.3 ± 5.24	80.3 [f] ± 1.66
12	76.9 ± 5.29	82.3 ± 5.29	79.9 ± 5.29	74.6 ± 5.29	76.1 ± 5.29	76.3 [g] ± 1.67
Yes						
1	96.6 ± 2.10	97.0 ± 2.10	96.1 ± 2.10	96.5 ± 2.10	93.9 ± 2.10	
2	93.7 ± 2.54	94.9 ± 2.54	94.9 ± 2.54	97.6 ± 2.54	93.0 ± 2.54	
3	96.4 ± 2.01	95.4 ± 2.01	92.9 ± 2.01	96.2 ± 2.01	91.8 ± 2.01	
4	94.0 ± 2.53	93.5 ± 2.53	88.0 ± 2.53	95.0 ± 2.53	89.4 ± 2.53	
5	90.6 ± 3.03	91.4 ± 3.03	87.0 ± 3.03	91.9 ± 3.03	86.0 ± 3.03	
6	90.0 ± 3.15	89.2 ± 3.15	83.6 ± 3.15	86.8 ± 3.15	82.7 ± 3.15	
7	87.6 ± 2.82	88.6 ± 2.82	79.5 ± 2.82	87.4 ± 2.82	82.3 ± 2.82	
8	88.9 ± 3.33	87.6 ± 3.33	79.8 ± 3.33	86.4 ± 3.33	85.5 ± 3.33	
9	87.3 ± 3.59	83.7 ± 3.59	77.6 ± 3.59	84.3 ± 3.59	85.5 ± 3.59	
10	79.6 ± 3.97	77.9 ± 3.97	81.4 ± 3.97	82.4 ± 3.97	76.4 ± 3.97	
11	78.8 ± 5.24	79.6 ± 5.24	78.1 ± 5.24	76.2 ± 5.24	81.0 ± 5.24	
12	76.8 ± 5.29	75.4 ± 5.29	73.8 ± 5.29	72.0 ± 5.29	74.8 ± 5.29	
Meal $\bar{x}$	87.7 ± 1.78	88.2 ± 1.78	87.4 ± 1.78	87.6 ± 1.78	86.6 ± 1.78	
Enzyme $\bar{x}$						
No			88.3 ± 1.12			
Yes			86.7 ± 1.12			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

a-g period means ± SEM with different superscripts are significantly different $\alpha \leq 0.05$

Table C.6. Three-way interaction means ± standard errors for feed conversion ratio (kg feed/kg egg mass) of brown-shell egg laying hens

Enzyme	Meal					
Period	SBM	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
1	1.99 ± 0.066	1.98 ± 0.061	1.95 ± 0.073	1.98 ± 0.066	1.89 ± 0.066	1.96 ± 0.021
2	2.02 ± 0.066	2.01 ± 0.061	1.95 ± 0.073	1.96 ± 0.066	1.87 ± 0.066	1.96 ± 0.021
3	1.99 ± 0.070	1.96 ± 0.061	1.85 ± 0.073	1.89 ± 0.066	1.88 ± 0.066	1.89 ± 0.021
4	1.97 ± 0.066	2.06 ± 0.061	1.93 ± 0.073	2.00 ± 0.066	1.95 ± 0.066	1.95 ± 0.021
5	2.11 ± 0.066	2.04 ± 0.061	1.94 ± 0.073	1.96 ± 0.070	1.96 ± 0.066	1.95 ± 0.021
6	2.05 ± 0.066	2.09 ± 0.061	2.03 ± 0.073	2.03 ± 0.066	1.96 ± 0.066	2.00 ± 0.021
7	1.93 ± 0.066	2.08 ± 0.061	1.97 ± 0.073	1.99 ± 0.066	1.90 ± 0.066	1.95 ± 0.021
8	1.98 ± 0.066	2.04 ± 0.061	1.99 ± 0.073	1.98 ± 0.066	1.98 ± 0.066	1.98 ± 0.021
9	2.01 ± 0.066	2.03 ± 0.061	2.00 ± 0.073	2.02 ± 0.066	1.91 ± 0.066	1.98 ± 0.021
10	2.06 ± 0.066	2.13 ± 0.061	2.08 ± 0.073	2.09 ± 0.066	1.96 ± 0.066	2.08 ± 0.021
11	2.19 ± 0.066	2.08 ± 0.061	2.10 ± 0.073	2.08 ± 0.066	1.95 ± 0.066	2.09 ± 0.021
12	2.22 ± 0.066	2.15 ± 0.061	2.18 ± 0.073	2.24 ± 0.066	2.19 ± 0.066	2.22 ± 0.021
Yes						
1	1.94 ± 0.066	1.95 ± 0.066	2.03 ± 0.066	1.96 ± 0.066	1.97 ± 0.066	
2	1.96 ± 0.066	1.92 ± 0.066	1.99 ± 0.066	1.93 ± 0.066	1.98 ± 0.066	
3	1.80 ± 0.066	1.91 ± 0.070	1.88 ± 0.066	1.87 ± 0.066	1.86 ± 0.066	
4	1.88 ± 0.066	1.90 ± 0.066	2.01 ± 0.066	1.92 ± 0.066	1.93 ± 0.066	
5	1.90 ± 0.066	1.86 ± 0.066	1.93 ± 0.066	1.91 ± 0.066	1.93 ± 0.070	
6	1.95 ± 0.066	1.98 ± 0.066	2.00 ± 0.066	1.91 ± 0.066	2.06 ± 0.066	
7	1.88 ± 0.070	1.86 ± 0.066	1.95 ± 0.066	1.95 ± 0.066	2.04 ± 0.070	
8	1.87 ± 0.066	1.95 ± 0.066	2.05 ± 0.066	1.91 ± 0.066	2.02 ± 0.066	
9	1.95 ± 0.066	1.96 ± 0.066	1.98 ± 0.066	1.94 ± 0.066	1.99 ± 0.066	
10	2.18 ± 0.066	2.18 ± 0.066	2.11 ± 0.066	1.91 ± 0.066	2.11 ± 0.066	
11	2.01 ± 0.070	2.18 ± 0.066	2.12 ± 0.070	1.99 ± 0.066	2.15 ± 0.066	
12	2.08 ± 0.070	2.32 ± 0.066	2.32 ± 0.066	2.20 ± 0.066	2.33 ± 0.066	
Meal $\bar{x}$	2.00 ± 0.033	2.02 ± 0.032	2.01 ± 0.035	1.98 ± 0.033	1.99 ± 0.033	
Enzyme $\bar{x}$						
No			2.01 ± 0.021			
Yes			1.99 ± 0.021			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Appendix D. Mortality data for white-and brown-shell egg laying hens.

Table D.1. Necropsy results of white-shell egg laying hens

Mortality #	Date (dd/mm/yyyy)	Cage #	Treatment	Cause / Notes
1	11/08/2010	85	3	Vent pecking / cannibalized Possible cage layer fatigue
2	16/08/2010	85	3	Prolapsed cloaca Soft ribs / possible cage layer fatigue (early)
3	21/08/2010	85	3	No necropsy completed
4	02/09/2010	25	9	Fatty liver hemorrhagic syndrome / Large blood clot over liver
5	02/09/2010	54	3	Trauma – head caught in feeder
6	02/09/2010	86	6	No diagnosis
7	20/09/2010	55	6	Head caught in feeder No necropsy completed
8	29/09/2010	52	10	No necropsy completed
9	29/09/2010	99	5	Culled – Osteoarthritis
10	29/09/2010	99	5	Culled – No necropsy completed
11	23/10/2010	37	7	Culled – Osteoporosis
12	27/10/2010	47	10	Prolapse / no necropsy completed
13	02/11/2010	50	5	*Staphylococcus aureus* acute infection Possible osteomalacia
14	12/11/2010	28	5	Suspected rickets
15	16/11/2010	43	7	No necropsy completed
16	24/11/2010	48	6	Culled – Suspected rickets
17	28/11/2010	91	1	Fatty liver syndrome
18	08/01/2011	47	10	Thin / blowout
19	16/01/2011	41	1	Prolapse / Fatty liver hemorrhagic syndrome
20	19/01/2011	26	7	Fatty liver hemorrhagic syndrome
21	26/01/2011	60	4	Apparent trauma to leg Egg bound
22	03/02/2011	81	8	No diagnosis
23	16/02/2011	44	4	Prolapsed cloaca
24	17/02/2011	42	9	No necropsy completed
25	29/03/2011	58	5	Prolapsed cloaca
26	16/04/2011	81	8	Culled – Suspected rickets
27	28/04/2011	44	4	Suspected rickets
28	29/04/2011	29	6	No diagnosis – Friable liver
29	09/05/2011	86	6	No diagnosis
30	24/05/2011	90	4	No diagnosis
31	27/05/2011	94	2	Fatty liver hemorrhagic syndrome
32	01/06/2011	55	6	No necropsy completed
33	03/07/2011	58	5	Soft bones / rickets
34	04/07/2011	37	7	Egg peritonitis

Table D.2. Necropsy results of brown-shell egg laying hens

Mortality #	Date (dd/mm/yyyy)	Cage #	Treatment	Cause / Notes
1	04/09/2010	98	5	Fatty liver hemorrhagic syndrome / large blood clot over liver
2	22/09/2010	96	8	Soft bones / possible rickets Breast blister
3	26/10/2010	36	8	Septicemia - *Staphylococcus aureus*
4	02/11/2010	31	4	Acute *E. coli* infection
5	28/11/2010	29	10	No diagnosis – small, pale yellow-orange liver
6	03/01/2011	86	8	Fatty liver hemorrhagic syndrome
7	17/01/2011	33	1	Fatty liver hemorrhagic syndrome
8	27/02/2011	87	9	Egg peritonitis
9	08/03/2011	96	8	Peck out / early fatty liver
10	05/04/2011	35	5	Prolapsed cloaca / early fatty liver
11	01/05/2011	22	4	Fatty liver hemorrhagic syndrome
12	02/05/2011	21	9	Septicemia - *Staphylococcus aureus*
13	05/05/2011	99	9	Hepatitis / Myocarditis Probable septicemia-*Staphylococcus aureus*
14	06/06/2011	47	2	Esophagitis
15	16/06/2011	48	3	No diagnosis

Appendix E. Three-way interaction tables for egg quality measurements on white-shell eggs.

Table E.1. Three-way interaction means ± standard errors for egg weight (g) of white-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	59.0 ± 0.78	58.4 ± 0.78	57.4 ± 0.78	58.5 ± 0.78	56.9 ± 0.78	57.9 ± 0.24
1	59.1 ± 0.72	60.2 ± 0.72	59.5 ± 0.72	60.0 ± 0.72	58.2 ± 0.72	59.0 ± 0.23
2	61.7 ± 0.94	60.0 ± 0.94	59.3 ± 0.94	60.0 ± 0.94	58.1 ± 0.94	59.7 ± 0.30
3	59.1 ± 0.87	57.9 ± 0.87	58.8 ± 0.87	59.6 ± 0.87	57.0 ± 0.87	58.4 ± 0.28
4	62.0 ± 0.93	60.9 ± 0.93	60.3 ± 0.93	60.8 ± 0.93	58.5 ± 0.93	60.0 ± 0.29
5	62.2 ± 0.79	61.1 ± 0.79	60.1 ± 0.79	60.2 ± 0.79	58.5 ± 0.79	60.5 ± 0.25
6	63.1 ± 1.06	61.8 ± 1.12	61.4 ± 1.12	62.7 ± 1.06	59.0 ± 1.06	61.5 ± 0.34
7	63.4 ± 0.79	61.1 ± 0.79	62.0 ± 0.79	62.6 ± 0.79	60.2 ± 0.79	61.4 ± 0.25
8	63.8 ± 0.89	63.1 ± 0.89	61.9 ± 0.89	63.2 ± 0.89	61.4 ± 0.89	62.3 ± 0.28
9	62.3 ± 0.93	62.8 ± 0.93	61.6 ± 0.93	63.0 ± 0.93	62.2 ± 0.93	62.3 ± 0.29
10	64.0 ± 0.96	63.7 ± 0.96	62.4 ± 0.95	63.7 ± 0.95	62.4 ± 0.95	62.7 ± 0.30
11	62.8 ± 1.01	63.3 ± 1.01	64.9 ± 1.01	63.1 ± 1.01	62. 3 ± 1.01	63.3 ± 0.32
12	62.4 ± 1.09	65.9 ± 1.09	64.1 ± 1.09	63.3 ± 1.09	62.4 ± 1.16	63.6 ± 0.35
Yes						
0	57.9 ± 0.78	57.8 ± 0.78	58.4 ± 0.78	57.0 ± 0.78	57.5 ± 0.78	
1	58.4 ± 0.72	59.0 ± 0.72	59.8 ± 0.72	57.8 ± 0.72	58.5 ± 0.72	
2	60.0 ± 0.94	58.2 ± 0.94	60.4 ± 0.94	60.1 ± 0.94	59.4 ± 0.94	
3	59.6 ± 0.87	56.6 ± 0.87	59.0 ± 0.87	58.5 ± 0.87	58.2 ± 0.87	
4	60.4 ± 0.93	57.5 ± 0.93	60.5 ± 0.93	59.8 ± 0.93	58.7 ± 0.93	
5	60.5 ± 0.79	61.2 ± 0.79	60.6 ± 0.79	60.9 ± 0.79	60.1 ± 0.79	
6	61.9 ± 1.06	60.7 ± 1.06	61.5 ± 1.06	61.5 ± 1.06	61.8 ± 1.06	
7	62.6 ± 0.79	61.2 ± 0.79	60.4 ± 0.79	61.7 ± 0.79	59.1 ± 0.79	
8	61.6 ± 0.89	61.6 ± 0.89	61.3 ± 0.89	63.1 ± 0.89	61.8 ± 0.89	
9	62.8 ± 0.93	61.7 ± 0.93	61.9 ± 0.93	64.3 ± 0.93	60.8 ± 0.93	
10	62.7 ± 0.95	60.5 ± 0.95	62.3 ± 0.95	63.8 ± 0.95	61.2 ± 0.95	
11	63.6 ± 1.01	63.1 ± 1.01	64.1 ± 1.01	63.6 ± 1.01	62.3 ± 1.01	
12	63.8 ± 1.09	63.9 ± 1.09	62.9 ± 1.16	65.2 ± 1.09	62.1 ± 1.09	
Meal $\bar{x}$	61.6 ± 0.41	60.9 ± 0.41	61.0 ± 0.42	61.4 ± 0.41	59.9 ± 0.41	
Enzyme $\bar{x}$						
No			61.2 ± 0.26			
Yes			60.8 ± 0.26			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table E.2. Three-way interaction means ± standard errors for percent yolk (% of egg weight) of white-shell eggs

Enzyme Period	Meal SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	29.5 ± 0.55	27.0 ± 0.55	27.4 ± 0.55	27.6 ± 0.55	26.8 ± 0.55	27.1 ± 0.17
1	29.0 ± 0.54	29.5 ± 0.54	29.4 ± 0.54	29.5 ± 0.54	29.2 ± 0.54	29.5 ± 0.17
2	28.6 ± 0.37	27.4 ± 0.37	26.9 ± 0.37	27.3 ± 0.37	26.9 ± 0.37	27.3 ± 0.12
3	28.6 ± 0.33	28.1 ± 0.33	28.1 ± 0.33	27.6 ± 0.33	27.9 ± 0.33	28.3 ± 0.10
4	29.3 ± 0.39	28.3 ± 0.39	27.9 ± 0.39	27.9 ± 0.39	27.2 ± 0.39	28.2 ± 0.12
5	29.7 ± 0.39	27.8 ± 0.39	28.1 ± 0.39	27.7 ± 0.39	26.6 ± 0.39	27.9 ± 0.12
6	29.1 ± 0.44	27.5 ± 0.47	27.2 ± 0.47	28.9 ± 0.44	27.5 ± 0.44	28.0 ± 0.14
7	29.8 ± 0.46	29.1 ± 0.46	28.5 ± 0.46	28.5 ± 0.46	27.3 ± 0.46	28.8 ± 0.15
8	28.8 ± 0.43	29.1 ± 0.43	28.4 ± 0.43	28.1 ± 0.43	27.5 ± 0.43	28.7 ± 0.14
9	28.9 ± 0.43	28.4 ± 0.43	27.9 ± 0.43	28.4 ± 0.43	27.4 ± 0.43	28.4 ± 0.14
10	27.0 ± 0.41	29.7 ± 0.41	28.5 ± 0.41	28.6 ± 0.41	27.9 ± 0.41	28.8 ± 0.13
11	30.3 ± 0.44	28.1 ± 0.44	27.7 ± 0.44	27.4 ± 0.44	27.7 ± 0.44	28.1 ± 0.14
12	27.9 ± 0.39	28.1 ± 0.39	27.6 ± 0.39	27.1 ± 0.42	27.1 ± 0.39	27.9 ± 0.12
Yes						
0	27.2 ± 0.55	29.0 ± 0.55	25.6 ± 0.55	27.1 ± 0.55	27.5 ± 0.55	
1	30.0 ± 0.54	27.3 ± 0.54	29.5 ± 0.54	30.0 ± 0.54	28.7 ± 0.54	
2	27.9 ± 0.37	28.8 ± 0.37	27.1 ± 0.37	27.7 ± 0.37	27.1 ± 0.37	
3	29.3 ± 0.33	28.9 ± 0.33	27.8 ± 0.33	28.2 ± 0.33	27.4 ± 0.33	
4	28.8 ± 0.39	28.0 ± 0.39	28.0 ± 0.39	28.0 ± 0.39	27.8 ± 0.39	
5	29.3 ± 0.39	28.4 ± 0.39	27.7 ± 0.39	28.0 ± 0.39	27.8 ± 0.39	
6	28.8 ± 0.44	29.3 ± 0.44	28.2 ± 0.44	28.0 ± 0.44	27.3 ± 0.44	
7	30.0 ± 0.46	29.4 ± 0.46	29.3 ± 0.46	28.4 ± 0.46	28.3 ± 0.46	
8	29.2 ± 0.43	28.6 ± 0.43	28.9 ± 0.43	29.2 ± 0.43	27.4 ± 0.43	
9	29.2 ± 0.43	29.0 ± 0.43	28.4 ± 0.43	28.4 ± 0.43	27.5 ± 0.43	
10	29.9 ± 0.41	28.4 ± 0.41	29.0 ± 0.41	28.5 ± 0.41	27.1 ± 0.41	
11	29.3 ± 0.44	28.4 ± 0.44	28.3 ± 0.44	28.4 ± 0.44	27.4 ± 0.44	
12	29.3 ± 0.39	29.0 ± 0.39	28.0 ± 0.42	27.9 ± 0.39	27.0 ± 0.39	
Meal $\bar{x}$	29.0 ± 0.16	28.4 ± 0.16	28.0 ± 0.16	28.2 ± 0.16	27.5 ± 0.16	
Enzyme $\bar{x}$						
No			28.1 ± 0.10			
Yes			28.3 ± 0.10			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table E.3. Three-way interaction means ± standard errors for percent albumen (% of egg weight) of white-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	63.1 ± 0.60	63.1 ± 0.60	62.7 ± 0.60	62.6 ± 0.60	63.2 ± 0.60	63.0 ± 0.19
1	59.5 ± 0.59	60.8 ± 0.59	60.9 ± 0.59	60.9 ± 0.59	61.1 ± 0.59	60.7 ± 0.19
2	62.3 ± 0.39	62.9 ± 0.39	63.3 ± 0.39	63.1 ± 0.39	63.3 ± 0.39	62.9 ± 0.12
3	60.4 ± 0.40	61.9 ± 0.40	61.9 ± 0.40	62.6 ± 0.40	61.9 ± 0.40	61.8 ± 0.13
4	61.4 ± 0.39	61.8 ± 0.39	62.2 ± 0.39	62.6 ± 0.39	62.9 ± 0.39	62.0 ± 0.12
5	61.8 ± 0.40	62.4 ± 0.40	62.2 ± 0.40	62.6 ± 0.40	63.8 ± 0.40	62.4 ± 0.13
6	61.7 ± 0.46	62.8 ± 0.49	63.5 ± 0.49	61.7 ± 0.46	62.9 ± 0.46	62.4 ± 0.15
7	61.3 ± 0.45	61.4 ± 0.45	62.0 ± 0.45	62.1 ± 0.45	63.1 ± 0.45	61.7 ± 0.14
8	61.1 ± 0.44	61.8 ± 0.44	62.1 ± 0.44	62.6 ± 0.44	63.2 ± 0.44	61.9 ± 0.14
9	61.5 ± 0.46	62.1 ± 0.46	62.7 ± 0.46	62.2 ± 0.46	63.2 ± 0.46	62.3 ± 0.15
10	61.0 ± 0.42	61.2 ± 0.42	62.2 ± 0.42	62.5 ± 0.42	62.8 ± 0.42	62.0 ± 0.13
11	61.7 ± 0.45	62.8 ± 0.45	63.1 ± 0.45	63.3 ± 0.45	63.1± 0.45	62.6 ± 0.14
12	62.0 ± 0.43	62.8 ± 0.43	63.2 ± 0.43	63.9 ± 0.46	63.7 ± 0.43	63.0 ± 0.14
Yes						
0	63.0 ± 0.60	62.6 ± 0.60	64.7 ± 0.60	63.0 ± 0.60	62.4 ± 0.60	
1	59.9 ± 0.59	61.3 ± 0.59	60.9 ± 0.59	60.0 ± 0.59	61.7 ± 0.59	
2	62.2 ± 0.39	62.8 ± 0.39	63.0 ± 0.39	62.9 ± 0.39	63.1± 0.39	
3	60.8 ± 0.40	61.2 ± 0.40	62.5 ± 0.40	61.9 ± 0.40	62.8 ± 0.40	
4	61.4 ± 0.39	61.2 ± 0.39	62.3 ± 0.39	62.2 ± 0.39	62.4 ± 0.39	
5	61.0 ± 0.41	62.5 ± 0.40	62.6 ± 0.40	62.4 ± 0.41	62.4 ± 0.40	
6	61.4 ± 0.46	62.1 ± 0.46	62.3 ± 0.46	62.4 ± 0.46	63.2 ± 0.46	
7	60.4 ± 0.45	61.0 ± 0.45	61.0 ± 0.45	62.3 ± 0.45	62.0 ± 0.45	
8	61.2 ± 0.44	61.2 ± 0.44	61.8 ± 0.44	61.5 ± 0.44	62.9 ± 0.44	
9	61.4 ± 0.46	62.2 ± 0.46	62.2 ± 0.46	62.2 ± 0.46	63.1 ± 0.46	
10	60.8 ± 0.42	61.5 ± 0.42	61.9 ± 0.42	62.2 ± 0.42	63.6 ± 0.42	
11	61.4 ± 0.45	62.4 ± 0.45	62.8 ± 0.45	62.2 ± 0.45	63.4 ± 0.45	
12	61.7 ± 0.43	62.6 ± 0.43	62.9 ± 0.46	63.0 ± 0.43	64.0 ± 0.43	
Meal $\bar{x}$	61.4 ± 0.18	62.0 ± 0.18	62.4 ± 0.18	62.3 ± 0.18	62.9 ± 0.18	
Enzyme $\bar{x}$						
No			62.3 ± 0.11			
Yes			62.1 ± 0.11			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table E.4. Three-way interaction means ± standard errors for percent shell (% of egg weight) of white-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	9.9 ± 0.14	9.9 ± 0.14	9.9 ± 0.14	9.8 ± 0.14	10.0 ± 0.14	9.9 ± 0.04
1	10.3 ± 0.14	9.7 ± 0.14	9.8 ± 0.14	9.7 ± 0.14	9.7 ± 0.14	9.8 ± 0.04
2	9.8 ± 0.14	9.7 ± 0.14	9.8 ± 0.14	9.7 ± 0.14	9.9 ± 0.14	9.8 ± 0.04
3	10.1± 0.14	10.1 ± 0.14	9.9 ± 0.14	9.9 ± 0.14	10.2 ± 0.14	10.0 ± 0.04
4	9.7± 0.14	9.8 ± 0.14	10.0 ± 0.14	9.6 ± 0.14	10.0 ± 0.14	9.8 ± 0.04
5	9.7 ± 0.14	9.8 ± 0.14	9.7 ± 0.14	9.7 ± 0.14	9.7 ± 0.14	9.7 ± 0.04
6	9.7 ± 0.14	9.7 ± 0.15	9.5 ± 0.15	9.4 ± 0.14	9.6 ± 0.14	9.6 ± 0.04
7	9.4 ± 0.14	9.6 ± 0.14	9.5 ± 0.14	9.5 ± 0.14	9.6 ± 0.14	9.6 ± 0.04
8	9.2 ± 0.14	9.2 ± 0.14	9.5 ± 0.14	9.3 ± 0.14	9.4 ± 0.14	9.4 ± 0.04
9	9.4 ± 0.14	9.4 ± 0.14	9.4 ± 0.14	9.4 ± 0.14	9.4 ± 0.14	9.4 ± 0.04
10	9.3 ± 0.14	9.2 ± 0.14	9.3 ± 0.14	9.0 ± 0.14	9.4 ± 0.14	9.3 ± 0.04
11	9.4 ± 0.14	9.2 ± 0.14	9.2 ± 0.14	9.2 ± 0.14	9.2 ± 0.14	9.2 ± 0.04
12	9.2 ± 0.14	9.1 ± 0.14	9.3 ± 0.14	9.0 ± 0.15	9.2 ± 0.14	9.1 ± 0.04
Yes						
0	9.8 ± 0.14	9.8 ± 0.14	9.9 ± 0.14	9.9 ± 0.14	10.0 ± 0.14	
1	10.1 ± 0.14	9.7 ± 0.14	9.6 ± 0.14	10.0 ± 0.14	9.6 ± 0.14	
2	10.0 ± 0.14	9.9 ± 0.14	9.9 ± 0.14	9.5 ± 0.14	9.8 ± 0.14	
3	9.9 ± 0.14	10.0 ± 0.14	9.8 ± 0.14	9.9 ± 0.14	9.9 ± 0.14	
4	9.8 ± 0.14	9.9 ± 0.14	9.8 ± 0.14	9.9 ± 0.14	9.8 ± 0.14	
5	9.7 ± 0.14	9.6 ± 0.14	9.7 ± 0.14	9.6 ± 0.14	9.7 ± 0.14	
6	9.7 ± 0.14	9.5 ± 0.14	9.6 ± 0.14	9.7 ± 0.14	9.5 ± 0.14	
7	9.7 ± 0.14	9.7 ± 0.14	9.6 ± 0.14	9.4 ± 0.14	9.7 ± 0.14	
8	9.6 ± 0.14	9.4 ± 0.14	9.4 ± 0.14	9.3 ± 0.14	9.7 ± 0.14	
9	9.4 ± 0.14	9.2 ± 0.14	9.4 ± 0.14	9.5 ± 0.14	9.4 ± 0.14	
10	9.2 ± 0.14	9.6 ± 0.14	9.2 ± 0.14	9.3 ± 0.14	9.4 ± 0.14	
11	9.3 ± 0.14	9.1 ± 0.14	9.0 ± 0.14	9.4 ± 0.14	9.2 ± 0.14	
12	9.0 ± 0.14	9.1 ± 0.14	9.1 ± 0.14	9.1 ± 0.14	9.0 ± 0.14	
Meal $\bar{x}$	9.6 ± 0.06	9.6 ± 0.06	9.6 ± 0.06	9.5 ± 0.06	9.6 ± 0.06	
Enzyme $\bar{x}$						
No			9.6 ± 0.04			
Yes			9.6 ± 0.04			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table E.5. Three-way interaction means ± standard errors for egg specific gravity of white-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	1.086 ± 9.99[2]	1.086 ± 9.99	1.089 ± 9.99	1.088 ± 9.99	1.088 ± 9.99	1.087 ± 3.16
1	1.083 ± 10.99	1.080 ± 10.99	1.081 ± 10.99	1.081 ± 10.99	1.081 ± 10.99	1.081 ± 3.48
2	1.089 ± 10.87	1.089 ± 10.87	1.089 ± 10.87	1.089 ± 10.87	1.090 ± 10.87	1.089 ± 3.44
3	1.087 ± 11.70	1.086 ± 11.70	1.087 ± 11.70	1.086 ± 11.70	1.087 ± 11.70	1.086 ± 3.70
4	1.085 ± 8.37	1.086 ± 8.37	1.086 ± 8.37	1.083 ± 8.37	1.087 ± 8.37	1.086 ± 2.65
5	1.084 ± 8.26	1.083 ± 8.26	1.083 ± 8.26	1.083 ± 8.26	1.083 ± 8.26	1.084 ± 2.61
6	1.084 ± 9.14	1.083 ± 9.83	1.083 ± 9.83	1.082 ± 9.14	1.083 ± 9.14	1.083 ± 2.94
7	1.078 ± 11.56	1.079 ± 11.56	1.080 ± 11.56	1.078 ± 11.56	1.079 ± 11.56	1.079 ± 3.66
8	1.080 ± 11.85	1.078 ± 11.85	1.080 ± 11.85	1.079 ± 11.85	1.079 ± 11.85	1.080 ± 3.75
9	1.081 ± 9.82	1.081 ± 9.82	1.082 ± 9.82	1.081 ± 9.82	1.083 ± 9.82	1.081 ± 3.11
10	1.080 ± 9.97	1.079 ± 9.97	1.080 ± 9.97	1.078 ± 9.97	1.080 ± 9.97	1.080 ± 3.15
11	1.081 ± 11.91	1.078 ± 11.91	1.079 ± 11.91	1.078 ± 11.91	1.079 ± 11.91	1.079 ± 3.77
12	1.080 ± 11.15	1.080 ± 11.15	1.082 ± 11.15	1.079 ± 12.12	1.081 ± 11.15	1.080 ± 3.59
Yes						
0	1.087 ± 9.99	1.087 ± 9.99	1.087 ± 9.99	1.088 ± 9.99	1.088 ± 9.99	
1	1.081 ± 10.99	1.081 ± 10.99	1.080 ± 10.99	1.081 ± 10.99	1.081 ± 10.99	
2	1.091 ± 10.87	1.090 ± 10.87	1.089 ± 10.87	1.088 ± 10.87	1.089 ± 10.87	
3	1.087 ± 11.70	1.087 ± 11.70	1.085 ± 11.70	1.087 ± 11.70	1.085 ± 11.70	
4	1.085 ± 8.37	1.087 ± 8.37	1.084 ± 8.37	1.086 ± 8.37	1.086 ± 8.37	
5	1.084 ± 8.26	1.083 ± 8.26	1.083 ± 8.26	1.083 ± 8.26	1.085 ± 8.26	
6	1.085 ± 9.14	1.083 ± 9.14	1.082 ± 9.14	1.083 ± 9.14	1.084 ± 9.14	
7	1.080 ± 11.56	1.080 ± 11.56	1.079 ± 11.56	1.078 ± 11.56	1.079 ± 11.56	
8	1.081 ± 11.85	1.080 ± 11.85	1.080 ± 11.85	1.079 ± 11.85	1.082 ± 11.85	
9	1.081 ± 9.82	1.079 ± 9.82	1.080 ± 9.82	1.082 ± 9.82	1.082 ± 9.82	
10	1.080 ± 9.97	1.081 ± 9.97	1.079 ± 9.97	1.079 ± 9.97	1.081 ± 9.97	
11	1.080 ± 11.91	1.078 ± 11.91	1.078 ± 11.91	1.079 ± 11.91	1.079 ± 11.91	
12	1.079 ± 11.15	1.081 ± 11.15	1.079 ± 12.12	1.079 ± 11.15	1.080 ± 11.15	
Meal $\bar{x}$	1.083 ± 2.87	1.083 ± 2.87	1.083 ± 2.88	1.083 ± 2.88	1.083 ± 2.87	
Enzyme $\bar{x}$						
No			1.083 ± 1.82			
Yes			1.083 ± 1.82			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

[2]All SEM are x 10^{-4}

Table E.6. Three-way interaction means ± standard errors for shell breaking strength (kg force) of white-shell eggs

Enzyme Period	Meal SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	5.31 ± 0.211	5.54 ± 0.211	5.44 ± 0.211	5.28 ± 0.211	5.37 ± 0.211	5.42 ± 0.067
1	5.56 ± 0.211	5.27 ± 0.211	5.40 ± 0.211	5.04 ± 0.211	5.22 ± 0.211	5.28 ± 0.067
2	5.38 ± 0.211	5.13 ± 0.211	5.14 ± 0.211	5.04 ± 0.211	5.15 ± 0.211	5.11 ± 0.067
3	4.33 ± 0.211	4.23 ± 0.211	4.19 ± 0.211	4.01 ± 0.211	3.91 ± 0.211	4.09 ± 0.067
4	5.48 ± 0.211	4.99 ± 0.211	5.33 ± 0.211	5.43 ± 0.211	5.72 ± 0.211	5.37 ± 0.067
5	5.52 ± 0.211	5.81 ± 0.211	5.40 ± 0.211	5.22 ± 0.211	5.33 ± 0.211	5.35 ± 0.067
6	3.36 ± 0.211	3.03 ± 0.229	2.60 ± 0.229	2.75 ± 0.211	2.73 ± 0.211	2.86 ± 0.068
7	5.15 ± 0.211	5.21 ± 0.211	5.24 ± 0.211	4.77 ± 0.211	5.02 ± 0.211	5.16 ± 0.067
8	5.02 ± 0.211	4.70 ± 0.211	4.99 ± 0.211	4.76 ± 0.211	4.93 ± 0.211	4.87 ± 0.067
9	4.80 ± 0.211	5.09 ± 0.211	4.92 ± 0.211	5.20 ± 0.211	5.08 ± 0.211	5.01 ± 0.067
10	4.74 ± 0.211	4.71 ± 0.211	4.76 ± 0.211	4.46 ± 0.211	4.46 ± 0.211	4.60 ± 0.067
11	4.40 ± 0.211	4.54 ± 0.211	4.50 ± 0.211	4.33 ± 0.211	3.96 ± 0.211	4.41 ± 0.067
12	4.68 ± 0.211	4.42 ± 0.211	4.42 ± 0.211	4.29 ± 0.211	4.69 ± 0.211	4.31 ± 0.067
Yes						
0	5.44 ± 0.211	5.59 ± 0.211	5.19 ± 0.211	5.63 ± 0.211	5.43 ± 0.211	
1	5.21 ± 0.211	5.35 ± 0.211	5.31 ± 0.211	5.24 ± 0.211	5.21 ± 0.211	
2	4.75 ± 0.211	5.13 ± 0.211	5.26 ± 0.211	4.81 ± 0.211	5.28 ± 0.211	
3	4.05 ± 0.211	4.08 ± 0.211	4.00 ± 0.211	4.21 ± 0.211	3.87 ± 0.211	
4	5.05 ± 0.211	5.41 ± 0.211	5.60 ± 0.211	5.45 ± 0.211	5.23 ± 0.211	
5	5.27 ± 0.211	5.19 ± 0.211	5.32 ± 0.211	5.35 ± 0.211	5.14 ± 0.211	
6	3.11 ± 0.211	2.79 ± 0.211	2.58 ± 0.211	2.94 ± 0.211	2.74 ± 0.211	
7	5.41 ± 0.211	5.26 ± 0.211	5.27 ± 0.211	4.94 ± 0.211	5.34 ± 0.211	
8	4.93 ± 0.211	4.79 ± 0.211	4.90 ± 0.211	4.76 ± 0.211	4.96 ± 0.211	
9	5.14 ± 0.211	4.93 ± 0.211	5.32 ± 0.211	4.86 ± 0.211	4.76 ± 0.211	
10	4.84 ± 0.211	4.47 ± 0.211	4.38 ± 0.211	4.56 ± 0.211	4.63 ± 0.211	
11	4.57 ± 0.211	4.58 ± 0.211	4.15 ± 0.211	4.47 ± 0.211	4.59 ± 0.211	
12	4.39 ± 0.211	4.13 ± 0.211	4.18 ± 0.229	4.08 ± 0.229	3.85 ± 0.211	
Meal $\bar{x}$	4.84 ± 0.065	4.78 ± 0.065	4.76 ± 0.065	4.69 ± 0.065	4.71 ± 0.065	
Enzyme $\bar{x}$						
No			4.78 ± 0.041			
Yes			4.73 ± 0.041			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table E.7. Three-way interaction means ± standard errors for albumen height (mm) of white-shell eggs

Enzyme Period	Meal SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0[2]	-	-	-	-	-	-
1	-	-	-	-	-	-
2	7.6 ± 0.19	7.6 ± 0.19	8.2 ± 0.19	8.1 ± 0.19	7.6 ± 0.19	7.8 ± 0.06
3	6.4 ± 0.19	6.4 ± 0.19	6.8 ± 0.19	6.8 ± 0.19	6.6 ± 0.19	6.6 ± 0.06
4	7.3 ± 0.19	6.9 ± 0.19	7.1 ± 0.19	7.6 ± 0.19	7.0 ± 0.19	7.2 ± 0.06
5	7.2 ± 0.19	6.8 ± 0.19	7.4 ± 0.19	7.6 ± 0.19	7.0 ± 0.19	7.2 ± 0.06
6	7.1 ± 0.19	6.9 ± 0.21	7.5 ± 0.21	7.4 ± 0.19	7.0 ± 0.19	7.1 ± 0.06
7	6.5 ± 0.19	6.5 ± 0.19	7.0 ± 0.19	6.9 ± 0.19	6.6 ± 0.19	6.8 ± 0.06
8	6.7 ± 0.19	6.8 ± 0.19	6.8 ± 0.19	6.7 ± 0.19	6.5 ± 0.19	6.8 ± 0.06
9	6.6 ± 0.19	6.6 ± 0.19	6.9 ± 0.19	7.3 ± 0.19	6.7 ± 0.19	6.8 ± 0.06
10	6.2 ± 0.19	6.3 ± 0.19	6.2 ± 0.19	6.6 ± 0.19	5.9 ± 0.19	6.2 ± 0.06
11	6.7 ± 0.19	6.3 ± 0.19	6.3 ± 0.19	6.6 ± 0.19	6.1 ± 0.19	6.3 ± 0.06
12	6.2 ± 0.19	6.3 ± 0.19	6.4 ± 0.19	6.6 ± 0.21	5.9 ± 0.19	6.3 ± 0.06
Yes						
0	-	-	-	-	-	
1	-	-	-	-	-	
2	7.7 ± 0.19	7.8 ± 0.19	8.2 ± 0.19	7.9 ± 0.19	7.9 ± 0.19	
3	6.4 ± 0.19	6.4 ± 0.19	6.7 ± 0.19	6.4 ± 0.19	7.0 ± 0.19	
4	7.4 ± 0.19	6.7 ± 0.19	7.5 ± 0.19	7.1 ± 0.19	7.4 ± 0.19	
5	7.2 ± 0.19	7.3 ± 0.19	7.4 ± 0.19	7.2 ± 0.19	7.2 ± 0.19	
6	6.9 ± 0.19	7.1 ± 0.19	7.3 ± 0.19	6.7 ± 0.19	7.6 ± 0.19	
7	6.8 ± 0.19	6.8 ± 0.19	6.7 ± 0.19	6.9 ± 0.19	7.0 ± 0.19	
8	6.9 ± 0.19	6.9 ± 0.19	6.9 ± 0.19	6.7 ± 0.19	6.7 ± 0.19	
9	7.0 ± 0.19	6.7 ± 0.19	7.0 ± 0.19	6.8 ± 0.19	6.7 ± 0.19	
10	6.3 ± 0.19	6.1 ± 0.19	6.1 ± 0.19	6.1 ± 0.19	6.3 ± 0.19	
11	6.4 ± 0.19	6.2 ± 0.19	6.2 ± 0.19	6.2 ± 0.19	6.1 ± 0.19	
12	6.4 ± 0.19	6.4 ± 0.19	6.0 ± 0.21	5.9 ± 0.19	6.4 ± 0.19	
Meal $\bar{x}$	6.8 ± 0.08	6.7 ± 0.08	6.9 ± 0.08	6.9 ± 0.08	6.7 ± 0.08	
Enzyme $\bar{x}$						
No			6.8 ± 0.05			
Yes			6.8 ± 0.05			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

[2]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12

Appendix F. Three-way interaction tables for egg quality measurements on brown-shell eggs.

Table F.1. Three-way interaction means ± standard errors for egg weight (g) of brown-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	58.3 ± 1.03	58.6 ± 0.95	59.1 ± 1.13	59.5 ± 1.03	59.9 ± 1.03	58.8 ± 0.33
1	61.3 ± 1.03	59.4 ± 0.95	59.1 ± 1.13	60.8 ± 1.03	61.5 ± 1.03	60.2 ± 0.33
2	62.1 ± 1.03	60.6 ± 0.95	60.6 ± 1.13	61.2 ± 1.03	62.5 ± 1.03	61.3 ± 0.33
3	62.2 ± 1.09	61.5 ± 0.95	60.9 ± 1.13	61.2 ± 1.03	60.6 ± 1.03	61.5 ± 0.33
4	63.2 ± 1.03	60.9 ± 0.95	60.3 ± 1.13	61.5 ± 1.03	60.5 ± 1.03	61.3 ± 0.33
5	63.0 ± 1.03	60.6 ± 0.95	60.7 ± 1.13	62.1 ± 1.09	60.4 ± 1.03	61.9 ± 0.33
6	64.2 ± 1.03	62.9 ± 0.95	62.1 ± 1.13	62.2 ± 1.03	63.2 ± 1.03	62.7 ± 0.33
7	64.5 ± 1.03	62.3 ± 0.95	62.5 ± 1.13	62.8 ± 1.03	63.4 ± 1.03	63.1 ± 0.33
8	64.9 ± 1.03	62.9 ± 0.95	62.3 ± 1.13	64.6 ± 1.03	62.2 ± 1.03	63.3 ± 0.33
9	65.2 ± 1.03	63.4 ± 0.95	63.4 ± 1.13	63.8 ± 1.03	63.8 ± 1.03	64.0 ± 0.33
10	64.2 ± 1.03	62.3 ± 0.95	63.3 ± 1.13	64.1 ± 1.03	64.5 ± 1.03	63.6 ± 0.33
11	63.6 ± 1.03	62.2 ± 0.95	63.7 ± 1.13	64.8 ± 1.03	63.8 ± 1.03	64.0 ± 0.33
12	64.8 ± 1.03	63.0 ± 0.95	64.9 ± 1.13	64.3 ± 1.03	63.8 ± 1.03	64.0 ± 0.33
Yes						
0	58.9 ± 1.03	58.8 ± 1.03	57.1 ± 1.03	60.5 ± 1.03	58.9 ± 1.03	
1	60.5 ± 1.03	59.8 ± 1.03	60.0 ± 1.03	61.7 ± 1.03	59.7 ± 1.03	
2	61.4 ± 1.03	62.0 ± 1.03	60.8 ± 1.03	62.2 ± 1.03	60.2 ± 1.03	
3	63.6 ± 1.03	61.3 ± 1.03	61.1 ± 1.03	61.2 ± 1.03	60.3 ± 1.03	
4	62.7 ± 1.03	61.4 ± 1.03	59.7 ± 1.03	62.0 ± 1.03	61.6 ± 1.03	
5	63.4 ± 1.03	62.7 ± 1.03	62.1 ± 1.03	64.0 ± 1.03	61.9 ± 1.09	
6	63.0 ± 1.03	61.9 ± 1.03	61.7 ± 1.03	62.6 ± 1.03	62.1 ± 1.03	
7	64.7 ± 1.09	64.0 ± 1.03	62.9 ± 1.03	64.3 ± 1.03	61.0 ± 1.09	
8	64.8 ± 1.03	63.2 ± 1.03	62.0 ± 1.03	64.5 ± 1.03	61.7 ± 1.03	
9	63.5 ± 1.03	65.0 ± 1.03	63.9 ± 1.03	63.9 ± 1.03	63.3 ± 1.03	
10	64.3 ± 1.03	62.4 ± 1.03	63.5 ± 1.03	65.8 ± 1.03	64.0 ± 1.03	
11	65.5 ± 1.03	62.6 ± 1.03	63.9 ± 1.03	65.0± 1.03	64.5 ± 1.03	
12	66.0 ± 1.03	62.6 ± 1.03	61.7 ± 1.03	60.5 ± 1.03	64.1 ± 1.03	
Meal $\bar{x}$	63.2 ± 0.52	61.8 ± 0.50	61.7 ± 0.55	62.7 ± 0.52	62.0 ± 0.52	
Enzyme $\bar{x}$						
No			62.3 ± 0.33			
Yes			62.3 ± 0.33			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table F.2. Three-way interaction means ± standard errors for percent yolk (% of egg weight) of brown-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	27.1 ± 0.61	26.8 ± 0.57	26.5 ± 0.67	26.9 ± 0.61	27.0 ± 0.61	26.7 ± 0.19
1	28.2 ± 0.67	28.7 ± 0.62	28.3 ± 0.73	28.9 ± 0.67	28.0 ± 0.67	28.1 ± 0.21
2	25.9 ± 0.38	26.1 ± 0.35	25.1 ± 0.41	25.5 ± 0.38	25.4 ± 0.38	25.5 ± 0.12
3	27.2 ± 0.53	26.6 ± 0.46	25.1 ± 0.54	26.6 ± 0.49	26.4 ± 0.49	26.5 ± 0.16
4	26.5 ± 0.39	26.8 ± 0.36	25.8 ± 0.43	26.5 ± 0.39	26.2 ± 0.39	26.3 ± 0.12
5	27.2 ± 0.40	26.7 ± 0.37	25.9 ± 0.43	26.1 ± 0.42	26.7 ± 0.40	26.4 ± 0.13
6	28.6 ± 0.51	28.3 ± 0.47	26.9 ± 0.55	27.6 ± 0.51	26.9 ± 0.51	27.6 ± 0.16
7	27.5 ± 0.57	28.6 ± 0.52	27.5 ± 0.62	28.2 ± 0.57	28.1 ± 0.57	27.7 ± 0.18
8	28.2 ± 0.43	28.2 ± 0.40	27.4 ± 0.47	27.1 ± 0.43	27.5 ± 0.43	27.8 ± 0.14
9	27.3 ± 0.36	27.5 ± 0.33	25.9 ± 0.39	26.7 ± 0.36	26.4 ± 0.36	26.9 ± 0.11
10	27.7 ± 0.40	27.7 ± 0.37	27.0 ± 0.43	27.0 ± 0.40	27.6 ± 0.40	27.2 ± 0.13
11	28.6 ± 0.48	28.2 ± 0.44	26.7 ± 0.52	27.6 ± 0.48	27.3 ± 0.48	27.4 ± 0.15
12	27.7 ± 0.42	27.5 ± 0.39	27.1 ± 0.47	27.3 ± 0.42	28.1 ± 0.42	27.5 ± 0.13
Yes						
0	26.5 ± 0.61	25.8 ± 0.61	27.7 ± 0.61	26.2 ± 0.61	26.8 ± 0.61	
1	27.9 ± 0.67	27.6 ± 0.72	28.1 ± 0.67	28.0 ± 0.67	27.8 ± 0.67	
2	25.3 ± 0.38	25.1 ± 0.38	25.5 ± 0.38	25.7 ± 0.38	25.1 ± 0.38	
3	26.4 ± 0.49	26.1 ± 0.53	27.6 ± 0.49	26.8 ± 0.49	26.0 ± 0.49	
4	26.5 ± 0.39	26.2 ± 0.39	26.5 ± 0.39	26.3 ± 0.39	26.0 ± 0.39	
5	26.4 ± 0.40	25.8 ± 0.40	26.4 ± 0.42	26.6 ± 0.40	26.2 ± 0.40	
6	27.5 ± 0.51	27.4 ± 0.51	27.9 ± 0.51	27.9 ± 0.51	27.2 ± 0.51	
7	27.4 ± 0.57	26.6 ± 0.57	27.7 ± 0.60	27.9 ± 0.60	27.3 ± 0.60	
8	28.9 ± 0.43	27.6 ± 0.43	28.1 ± 0.43	27.6 ± 0.43	27.6 ± 0.43	
9	27.2 ± 0.36	26.7 ± 0.36	27.5 ± 0.36	26.8 ± 0.36	26.5 ± 0.36	
10	27.6 ± 0.40	27.3 ± 0.40	27.1 ± 0.40	27.1 ± 0.40	26.0 ± 0.40	
11	27.3 ± 0.48	27.4 ± 0.48	26.5 ± 0.48	27.3 ± 0.48	26.7 ± 0.48	
12	27.7 ± 0.43	27.3 ± 0.42	27.8 ± 0.42	27.3 ± 0.42	27.3 ± 0.42	
Meal $\bar{x}$	27.3 ± 0.20	27.1 ± 0.20	26.9 ± 0.21	27.0 ± 0.20	26.8 ± 0.20	
Enzyme $\bar{x}$						
No			27.1 ± 0.13			
Yes			26.9 ± 0.13			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table F.3. Three-way interaction means ± standard errors for percent albumen (% of egg weight) of brown-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	63.4 ± 0.69	63.4 ± 0.64	63.5 ± 0.76	63.3 ± 0.69	63.8 ± 0.69	63.4 ± 0.22
1	62.3 ± 0.58	62.3 ± 0.57	62.7 ± 0.69	61.6 ± 0.58	64.4 ± 0.58	62.3 ± 0.19
2	64.4 ± 0.41	63.8 ± 0.38	64.8 ± 0.45	64.5 ± 0.41	62.5 ± 0.41	64.7 ± 0.13
3	62.8 ± 0.54	63.5 ± 0.47	64.6 ± 0.56	63.4 ± 0.51	63.4 ± 0.51	63.6 ± 0.16
4	63.6 ± 0.43	63.0 ± 0.40	64.0 ± 0.47	63.6 ± 0.43	63.8 ± 0.43	63.7 ± 0.14
5	63.2 ± 0.42	63.4 ± 0.39	64.0 ± 0.46	63.9 ± 0.45	63.6 ± 0.42	63.7 ± 0.14
6	61.5 ± 0.51	61.9 ± 0.47	63.2 ± 0.56	62.5 ± 0.51	63.5 ± 0.51	62.6 ± 0.16
7	62.8 ± 0.63	61.6 ± 0.58	62.3 ± 0.69	62.1 ± 0.63	62.3 ± 0.63	62.5 ± 0.20
8	62.3 ± 0.46	61.8 ± 0.42	62.4 ± 0.50	63.0 ± 0.46	62.7 ± 0.46	62.3 ± 0.14
9	63.2 ± 0.42	62.8 ± 0.39	64.0 ± 0.46	63.5 ± 0.42	64.0 ± 0.42	63.4 ± 0.13
10	63.1 ± 0.45	62.3 ± 0.41	63.0 ± 0.49	63.4 ± 0.45	62.8 ± 0.45	63.1 ± 0.14
11	62.0 ± 0.53	62.1 ± 0.49	63.1 ± 0.58	62.8 ± 0.53	63.5 ± 0.53	63.0 ± 0.17
12	62.6 ± 0.45	62.9 ± 0.42	63.0 ± 0.50	63.4 ± 0.45	62.6 ± 0.45	62.9 ± 0.14
Yes						
0	63.8 ± 0.69	63.8 ± 0.69	62.4 ± 0.69	64.0 ± 0.69	63.4 ± 0.69	
1	62.1 ± 0.58	64.4 ± 0.62	62.3 ± 0.58	62.2 ± 0.58	62.4 ± 0.58	
2	64.8 ± 0.41	62.5 ± 0.41	64.7 ± 0.41	64.5 ± 0.41	65.2 ± 0.41	
3	63.7 ± 0.51	63.4 ± 0.54	63.0 ± 0.51	63.3 ± 0.51	64.0 ± 0.51	
4	63.3 ± 0.43	62.2 ± 0.43	63.6 ± 0.43	63.7 ± 0.43	64.2 ± 0.43	
5	63.8 ± 0.42	64.2 ± 0.42	63.9 ± 0.42	63.2 ± 0.42	63.8 ± 0.45	
6	62.8 ± 0.51	62.6 ± 0.51	62.5 ± 0.51	62.2 ± 0.51	63.1 ± 0.51	
7	62.6 ± 0.67	65.0 ± 0.63	62.9 ± 0.63	62.1 ± 0.67	62.6 ± 0.67	
8	61.4 ± 0.46	64.2 ± 0.46	62.4 ± 0.46	62.5 ± 0.46	62.7 ± 0.46	
9	62.6 ± 0.42	64.2 ± 0.42	62.9 ± 0.42	63.2 ± 0.42	63.9 ± 0.42	
10	62.5 ± 0.45	62.6 ± 0.45	63.3 ± 0.45	63.2 ± 0.45	64.7 ± 0.45	
11	62.8 ± 0.53	65.0 ± 0.53	64.0 ± 0.53	63.1 ± 0.53	63.8 ± 0.53	
12	62.6 ± 0.45	64.2 ± 0.45	62.8 ± 0.45	63.2 ± 0.45	63.1 ± 0.45	
Meal $\bar{x}$	62.9 ± 0.22	63.1 ± 0.22	63.3 ± 0.23	63.1 ± 0.22	63.4 ± 0.22	
Enzyme $\bar{x}$						
No			63.1 ± 0.14			
Yes			63.2 ± 0.14			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table F.4. Three-way interaction means ± standard errors for percent shell (% of egg weight) of brown-shell eggs

Enzyme Period	Meal SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	9.5 ± 0.20	9.8 ± 0.18	10.0 ± 0.22	9.8 ± 0.20	10.0 ± 0.20	9.8 ± 0.06
1	9.6 ± 0.18	9.9 ± 0.17	10.2 ± 0.20	9.6 ± 0.18	9.6 ± 0.18	9.8 ± 0.06
2	9.7 ± 0.13	10.1 ± 0.12	10.2 ± 0.14	10.0 ± 0.13	9.7 ± 0.13	9.9 ± 0.04
3	10.0 ± 0.14	9.9 ± 0.12	10.2 ± 0.14	10.0 ± 0.13	10.2 ± 0.13	9.9 ± 0.04
4	9.9 ± 0.14	10.1 ± 0.13	10.3 ± 0.15	9.8 ± 0.14	10.0 ± 0.14	10.0 ± 0.04
5	9.7 ± 0.13	10.0 ± 0.12	10.2 ± 0.15	9.9 ± 0.15	9.7 ± 0.13	9.9 ± 0.04
6	9.9 ± 0.14	9.9 ± 0.13	9.9 ± 0.15	9.9 ± 0.14	9.6 ± 0.14	9.8 ± 0.04
7	9.7 ± 0.15	9.9 ± 0.14	10.2 ± 0.17	9.8 ± 0.15	9.7 ± 0.15	9.9 ± 0.05
8	9.4 ± 0.18	10.0 ± 0.16	10.2 ± 0.19	9.8 ± 0.18	9.8 ± 0.18	9.8 ± 0.06
9	9.5 ± 0.22	9.7 ± 0.20	10.1 ± 0.24	9.8 ± 0.22	9.6 ± 0.22	9.8 ± 0.07
10	9.6 ± 0.15	9.9 ± 0.14	10.0 ± 0.17	9.7 ± 0.15	9.6 ± 0.15	9.7 ± 0.05
11	9.7 ± 0.18	9.7 ± 0.17	10.0 ± 0.20	9.7 ± 0.18	9.3 ± 0.18	9.7 ± 0.06
12	9.7 ± 0.14	9.6 ± 0.13	9.9 ± 0.15	9.4 ± 0.14	9.4 ± 0.14	9.6 ± 0.04
Yes						
0	9.8 ± 0.20	10.0 ± 0.20	9.7 ± 0.19	10.1 ± 0.21	9.8 ± 0.20	
1	10.0 ± 0.18	10.0 ± 0.18	9.7 ± 0.18	9.9 ± 0.18	9.8 ± 0.18	
2	10.0 ± 0.13	9.9 ± 0.13	9.8 ± 0.13	9.9 ± 0.13	9.7 ± 0.13	
3	9.9 ± 0.13	9.8 ± 0.14	9.4 ± 0.13	9.9 ± 0.13	10.0 ± 0.13	
4	10.3 ± 0.14	10.0 ± 0.14	9.9 ± 0.14	10.1 ± 0.14	9.9 ± 0.14	
5	9.9 ± 0.13	9.8 ± 0.14	9.7 ± 0.13	10.2 ± 0.13	10.0 ± 0.15	
6	9.9 ± 0.14	10.1 ± 0.14	9.6 ± 0.14	10.0 ± 0.14	9.7 ± 0.14	
7	10.0 ± 0.17	10.0 ± 0.15	9.4 ± 0.15	10.0 ± 0.15	9.9 ± 0.17	
8	9.8 ± 0.18	10.1 ± 0.18	9.5 ± 0.18	9.9 ± 0.18	9.8 ± 0.18	
9	10.2 ± 0.22	9.9 ± 0.22	9.6 ± 0.22	10.1 ± 0.22	9.6 ± 0.22	
10	10.0 ± 0.15	9.8 ± 0.15	9.6 ± 0.15	9.7 ± 0.15	9.4 ± 0.15	
11	9.8 ± 0.18	10.0 ± 0.18	9.5 ± 0.18	9.7 ± 0.18	9.6 ± 0.18	
12	9.7 ± 0.14	9.7 ± 0.14	9.5 ± 0.14	9.8 ± 0.14	9.8 ± 0.14	
Meal $\bar{x}$	9.8 ± 0.07	9.9 ± 0.06	9.9 ± 0.07	9.9 ± 0.07	9.7 ± 0.07	
Enzyme $\bar{x}$						
No			9.8 ± 0.04			
Yes			9.8 ± 0.04			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table F.5. Three-way interaction means ± standard errors for specific gravity of brown-shell eggs						
Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	1.086 ± 12.61[2]	1.090 ± 11.67	1.090 ± 13.81	1.087 ± 12.61	1.087 ± 12.61	1.088 ± 4.00
1	1.082 ± 10.68	1.084 ± 9.89	1.085 ± 11.70	1.083 ± 10.68	1.081 ± 10.68	1.082 ± 3.39
2	1.090 ± 9.37	1.093 ± 8.67	1.093 ± 10.26	1.093 ± 9.37	1.089 ± 9.37	1.091 ± 2.97
3	1.089 ± 10.58	1.088 ± 9.20	1.089 ± 10.88	1.089 ± 9.94	1.088 ± 9.94	1.088 ± 3.19
4	1.088 ± 10.82	1.089 ± 10.01	1.089 ± 11.85	1.087 ± 10.82	1.087 ± 10.82	1.088 ± 3.43
5	1.086 ± 11.02	1.087 ± 9.45	1.088 ± 11.81	1.086 ± 11.02	1.085 ± 10.21	1.087 ± 3.29
6	1.088 ± 8.91	1.088 ± 8.25	1.088 ± 9.76	1.088 ± 8.91	1.085 ± 8.91	1.087 ± 2.83
7	1.083 ± 12.00	1.085 ± 11.11	1.086 ± 13.14	1.084 ± 12.00	1.082 ± 12.00	1.084 ± 3.87
8	1.085 ± 11.98	1.087 ± 11.09	1.087 ± 13.12	1.084 ± 11.98	1.084 ± 11.98	1.085 ± 3.80
9	1.085 ± 14.02	1.086 ± 12.98	1.088 ± 15.35	1.085 ± 14.02	1.084 ± 14.02	1.086 ± 4.45
10	1.083 ± 11.21	1.086 ± 10.38	1.087 ± 12.28	1.084 ± 11.21	1.083 ± 11.21	1.084 ± 3.56
11	1.084 ± 12.93	1.085 ± 11.97	1.086 ± 14.16	1.085 ± 12.93	1.082 ± 12.93	1.084 ± 4.10
12	1.085 ± 10.84	1.085 ± 10.04	1.086 ± 11.88	1.084 ± 10.84	1.082 ± 10.84	1.084 ± 3.44
Yes						
0	1.087 ± 12.61	1.088 ± 12.61	1.086 ± 12.61	1.086 ± 12.61	1.088 ± 12.61	
1	1.083 ± 10.68	1.082 ± 10.68	1.080 ± 10.68	1.082 ± 10.68	1.081 ± 10.68	
2	1.092 ± 9.37	1.091 ± 9.37	1.091 ± 9.37	1.091 ± 9.37	1.090 ± 9.37	
3	1.088 ± 9.94	1.087 ± 10.58	1.084 ± 9.94	1.088 ± 9.44	1.088 ± 9.44	
4	1.090 ± 10.82	1.087 ± 10.82	1.087 ± 10.82	1.089 ± 10.82	1.086 ± 10.82	
5	1.087± 10.21	1.087 ± 10.21	1.086 ± 10.21	1.088 ± 10.21	1.087 ± 11.02	
6	1.088 ± 8.91	1.089 ± 8.91	1.086 ± 8.91	1.088 ± 8.91	1.086 ± 8.91	
7	1.085 ± 13.05	1.085 ± 12.00	1.080 ± 12.00	1.085 ± 12.00	1.085 ± 13.05	
8	1.085 ± 11.98	1.089 ± 11.98	1.082 ± 11.98	1.086 ± 11.98	1.084 ± 11.98	
9	1.089 ± 14.02	1.086 ± 14.02	1.083 ± 14.02	1.087 ± 14.02	1.083 ± 14.02	
10	1.086 ± 11.21	1.086 ± 11.21	1.083 ± 11.21	1.084 ± 11.21	1.082 ± 11.21	
11	1.085 ± 12.93	1.086 ± 12.93	1.083 ± 12.93	1.084 ± 12.93	1.083 ± 12.93	
12	1.085 ± 10.84	1.084 ± 10.84	1.083 ± 10.84	1.085 ± 10.84	1.084 ± 10.84	
Meal $\bar{x}$	1.086 ± 3.13	1.087 ± 3.01	1.086 ± 3.27	1.086 ± 3.12	1.085 ± 3.13	
Enzyme $\bar{x}$						
No			1.086 ± 1.98			
Yes			1.086 ± 1.98			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

[2]All SEM are x 10^{-4}

Table F.6. Three-way interaction means ± standard errors for shell breaking strength (kg force) of brown-shell eggs

Enzyme Period	Meal SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0	5.37 ± 0.239	5.66 ± 0.221	5.44 ± 0.261	5.73 ± 0.239	5.54 ± 0.239	5.54 ± 0.076
1	5.44 ± 0.239	5.58 ± 0.221	5.41 ± 0.261	5.22 ± 0.239	5.50 ± 0.239	5.42 ± 0.076
2	5.15 ± 0.239	5.41 ± 0.221	5.48 ± 0.261	5.43 ± 0.239	5.38 ± 0.239	5.35 ± 0.076
3	4.76 ± 0.257	4.32 ± 0.221	4.56 ± 0.261	5.00 ± 0.239	4.51 ± 0.239	4.42 ± 0.077
4	5.79 ± 0.239	5.34 ± 0.221	5.44 ± 0.261	4.58 ± 0.239	6.02 ± 0.239	5.72 ± 0.076
5	5.55 ± 0.239	5.45 ± 0.221	5.81 ± 0.261	5.88 ± 0.257	5.69 ± 0.239	5.54 ± 0.077
6	3.60 ± 0.239	3.53 ± 0.221	3.39 ± 0.261	5.28 ± 0.239	3.13 ± 0.239	3.43 ± 0.076
7	4.92 ± 0.239	5.64 ± 0.221	5.67 ± 0.261	5.83 ± 0.239	5.51 ± 0.239	5.38 ± 0.077
8	5.17 ± 0.239	5.66 ± 0.221	5.75 ± 0.261	4.72 ± 0.257	5.47 ± 0.239	5.46 ± 0.076
9	5.12 ± 0.239	5.51 ± 0.221	5.77 ± 0.261	5.81 ± 0.239	5.34 ± 0.239	5.24 ± 0.076
10	4.99 ± 0.239	5.36 ± 0.221	5.13 ± 0.261	5.54 ± 0.239	5.29 ± 0.239	5.15 ± 0.076
11	4.56 ± 0.239	4.84 ± 0.221	4.82 ± 0.323	3.53 ± 0.239	4.42 ± 0.239	4.73 ± 0.078
12	4.88 ± 0.239	4.50 ± 0.221	4.54 ± 0.261	5.53 ± 0.239	4.29 ± 0.239	4.58 ± 0.076
Yes						
0	5.52 ± 0.239	5.56 ± 0.239	4.85 ± 0.239	5.86 ± 0.239	5.73 ± 0.239	
1	5.36 ± 0.239	5.46 ± 0.239	5.33 ± 0.239	5.49 ± 0.239	5.33 ± 0.239	
2	5.06 ± 0.239	5.52 ± 0.239	5.07 ± 0.239	5.31 ± 0.239	5.26 ± 0.239	
3	4.20 ± 0.239	4.37 ± 0.257	3.55 ± 0.239	4.62 ± 0.239	4.56 ± 0.239	
4	5.78 ± 0.239	6.00 ± 0.239	5.67 ± 0.239	5.63 ± 0.239	5.78 ± 0.239	
5	5.35 ± 0.239	5.50 ± 0.239	5.58 ± 0.239	5.33 ± 0.239	5.60 ± 0.257	
6	3.31 ± 0.239	3.72 ± 0.239	3.11 ± 0.239	3.67 ± 0.239	3.34 ± 0.239	
7	5.40 ± 0.257	5.66 ± 0.239	4.68 ± 0.239	5.46 ± 0.239	5.37 ± 0.257	
8	5.32 ± 0.239	5.65 ± 0.239	5.31 ± 0.239	5.30 ± 0.239	5.25 ± 0.239	
9	5.13 ± 0.239	5.47 ± 0.239	4.84 ± 0.239	5.11 ± 0.239	4.96 ± 0.239	
10	5.04 ± 0.239	5.22 ± 0.239	5.11 ± 0.257	4.82 ± 0.239	5.10 ± 0.239	
11	4.78 ± 0.239	4.84 ± 0.239	4.54 ± 0.239	4.74 ± 0.239	4.82 ± 0.239	
12	4.46 ± 0.239	4.69 ± 0.239	4.34 ± 0.239	4.68 ± 0.239	4.79 ± 0.239	
Meal $\bar{x}$	5.0 ± 0.095	5.17 ± 0.092	4.97 ± 0.100	5.16 ± 0.095	5.08 ± 0.095	
Enzyme $\bar{x}$						
No			5.13 ± 0.061			
Yes			5.02 ± 0.060			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean

Table F.7. Three-way interaction means ± standard errors for albumen height (mm) of brown-shell eggs

Enzyme	Meal					
Period	SBM[1]	10 % CM	20 % CM	10 % JM	20 % JM	Period $\bar{x}$
No						
0[2]	-	-	-	-	-	-
1	-	-	-	-	-	-
2	7.1 ± 0.24	7.1 ± 0.22	6.8 ± 0.26	7.1 ± 0.24	7.2 ± 0.24	7.0 ± 0.08
3	6.6 ± 0.26	7.1 ± 0.22	6.7 ± 0.26	6.8 ± 0.24	6.9 ± 0.24	6.9 ± 0.08
4	6.2 ± 0.24	5.8 ± 0.22	6.3 ± 0.26	6.0 ± 0.24	5.8 ± 0.24	5.9 ± 0.08
5	6.1 ± 0.24	6.0 ± 0.22	5.9 ± 0.26	5.7 ± 0.26	6.1 ± 0.24	6.0 ± 0.08
6	5.9 ± 0.24	5.6 ± 0.22	5.5 ± 0.26	5.2 ± 0.24	5.4 ± 0.24	5.5 ± 0.08
7	5.2 ± 0.24	5.4 ± 0.22	5.0 ± 0.26	5.2 ± 0.24	4.9 ± 0.24	5.2 ± 0.08
8	5.8 ± 0.24	5.4 ± 0.22	5.6 ± 0.26	5.5 ± 0.24	5.4 ± 0.24	5.5 ± 0.08
9	5.9 ± 0.24	5.7 ± 0.22	5.9 ± 0.26	5.4 ± 0.24	5.8 ± 0.24	5.7 ± 0.08
10	4.8 ± 0.24	4.7 ± 0.22	4.7 ± 0.26	4.7 ± 0.24	4.7 ± 0.24	4.8 ± 0.08
11	5.0 ± 0.24	5.1 ± 0.22	5.1 ± 0.26	4.9 ± 0.24	5.1 ± 0.24	5.0 ± 0.08
12	5.0 ± 0.24	5.5 ± 0.22	5.1 ± 0.26	5.0 ± 0.24	5.4 ± 0.24	5.2 ± 0.08
Yes						
0	-	-	-	-	-	
1	-	-	-	-	-	
2	7.5 ± 0.24	7.2 ± 0.24	7.1 ± 0.24	6.6 ± 0.24	6.8 ± 0.24	
3	7.0 ± 0.24	6.9 ± 0.26	7.0 ± 0.24	6.8 ± 0.24	6.6 ± 0.24	
4	6.1 ± 0.24	5.7 ± 0.24	6.0 ± 0.24	5.9 ± 0.24	5.5 ± 0.24	
5	6.3 ± 0.24	5.9 ± 0.24	6.3 ± 0.24	5.8 ± 0.24	5.8 ± 0.26	
6	5.6 ± 0.24	5.5 ± 0.24	5.9 ± 0.24	5.4 ± 0.24	5.0 ± 0.24	
7	5.4 ± 0.26	5.3 ± 0.24	5.2 ± 0.24	5.0 ± 0.24	5.4 ± 0.26	
8	5.6 ± 0.24	5.5 ± 0.24	5.6 ± 0.24	5.2 ± 0.24	5.5 ± 0.24	
9	5.6 ± 0.24	5.9 ± 0.24	6.0 ± 0.24	5.6 ± 0.24	5.4 ± 0.24	
10	5.2 ± 0.24	5.1 ± 0.24	5.3 ± 0.24	4.6 ± 0.24	4.8 ± 0.24	
11	5.3 ± 0.24	5.0 ± 0.24	5.2 ± 0.24	4.6 ± 0.24	5.0 ± 0.24	
12	5.5 ± 0.24	5.2 ± 0.24	5.2 ± 0.24	5.0 ± 0.24	4.8 ± 0.24	
Meal $\bar{x}$	5.8 ± 0.10	5.8 ± 0.10	5.8 ± 0.11	5.5 ± 0.10	5.6 ± 0.10	
Enzyme $\bar{x}$						
No			5.7 ± 0.07			
Yes			5.7 ± 0.07			

[1]SBM = soybean meal, CM = canola meal, JM = juncea meal, $\bar{x}$ = mean
[2]Period 0 and period 1 removed from statistical analysis due to prolonged storage compared to periods 2-12

Appendix G. Data for QCT and quality measurements of humeri of brown-shell egg laying hens.

Table G.1. ANOVA *P*-values for humerus cross-sectional area measurements from brown-shell egg laying hens

	Area		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.9755	0.6546	0.9679
Meal	0.1262	0.1934	0.2370
Enzyme*Meal	0.1503	0.4153	0.1704

Table G.2. Humerus cross-sectional area measurements from brown-shell egg laying hens

	Total Area (mm^2)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	62.68 ± 1.818	64.12 ± 1.818	63.40 ± 1.286
10 % Canola Meal	68.45 ± 1.818	62.73 ± 1.818	65.59 ± 1.286
20 % Canola Meal	60.87 ± 1.818	63.83 ± 1.818	62.35 ± 1.286
10 % Juncea Meal	65.66 ± 1.818	67.25 ± 1.818	66.46 ± 1.286
20 % Juncea Meal	65.86 ± 1.818	65.78 ± 1.818	65.82 ± 1.286
Enzyme Means	64.70 ± 0.813	64.74 ± 0.813	
	Cortical Area (mm^2)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	14.27 ± 0.405	13.74 ± 0.405	14.00 ± 0.286
10 % Canola Meal	14.40 ± 0.405	13.86 ± 0.405	14.13 ± 0.286
20 % Canola Meal	13.75 ± 0.405	14.45 ± 0.405	14.10 ± 0.286
10 % Juncea Meal	14.26 ± 0.405	14.54 ± 0.405	14.40 ± 0.286
20 % Juncea Meal	15.13 ± 0.405	14.64 ± 0.405	14.89 ± 0.286
Enzyme Means	14.36 ± 0.181	14.25 ± 0.181	
	Trabecular Area (mm^2)		
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	47.85 ± 1.732	49.47 ± 1.732	48.66 ± 1.225
10 % Canola Meal	53.54 ± 1.732	47.95 ± 1.732	50.75 ± 1.225
20 % Canola Meal	46.66 ± 1.732	48.76 ± 1.732	47.71 ± 1.225
10 % Juncea Meal	50.73 ± 1.732	51.69 ± 1.732	51.21 ± 1.225
20 % Juncea Meal	49.66 ± 1.732	50.35 ± 1.732	50.01 ± 1.225
Enzyme Means	49.69 ± 0.775	49.64 ± 0.775	

Table G.3. ANOVA *P*-values for humerus density measurements from brown-shell egg laying hens

	Density	
	Total	Cortical
Source of Variation		
Enzyme	0.6057	0.2621
Meal	0.7703	0.9194
Enzyme*Meal	0.4129	0.4775

Table G.4. Humerus density measurements from brown-shell egg laying hens

Total Density (mg/cm^3)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	173.6 ± 21.58	131.4 ± 21.58	149.5 ± 15.12
10 % Canola Meal	142.9 ± 21.58	143.7 ± 21.58	143.3 ± 15.12
20 % Canola Meal	144.7 ± 21.58	174.8 ± 21.58	158.2 ± 15.12
10 % Juncea Meal	147.7 ± 21.58	142.2 ± 21.58	144.9 ± 15.12
20 % Juncea Meal	168.1 ± 21.58	158.0 ± 21.58	162.9 ± 15.12
Enzyme Means	154.3 ± 9.36	148.6 ± 9.36	
Cortical Density (mg/cm^3)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	1046.6 ± 10.54	1036.7 ± 10.54	1041.6 ± 7.46
10 % Canola Meal	1043.0 ± 10.54	1037.4 ± 10.54	1040.2 ± 7.46
20 % Canola Meal	1050.4 ± 10.54	1032.3 ± 10.54	1041.4 ± 7.46
10 % Juncea Meal	1043.1 ± 10.54	1023.7 ± 10.54	1033.4 ± 7.46
20 % Juncea Meal	1028.6 ± 10.54	1044.0 ± 10.54	1036.3 ± 7.46
Enzyme Means	1042.3 ± 4.72	1034.8 ± 4.72	

Table G.5. ANOVA *P*-values for humerus bone mineral content measurements from brown-shell egg laying hens

	Bone Mineral Content	
	Total	Cortical
Source of Variation		
Enzyme	0.5494	0.2621
Meal	0.7806	0.9194
Enzyme*Meal	0.3373	0.4775

Table G.6. Humerus bone mineral content measurements from brown-shell egg laying hens

Total Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	10.96 ± 1.288	8.51 ± 1.288	9.58 ± 0.911
10 % Canola Meal	9.76 ± 1.288	8.98 ± 1.288	9.35 ± 0.911
20 % Canola Meal	8.89 ± 1.288	10.96 ± 1.288	9.81 ± 0.911
10 % Juncea Meal	9.87 ± 1.288	9.52 ± 1.288	9.70 ± 0.911
20 % Juncea Meal	10.96 ± 1.288	10.53 ± 1.288	10.74 ± 0.911
Enzyme Means	10.03 ± 0.576	9.62 ± 0.576	
Cortical Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	14.94 ± 0.436	14.24 ± 0.436	14.59 ± 0.308
10 % Canola Meal	15.02 ± 0.436	14.38 ± 0.436	14.70 ± 0.308
20 % Canola Meal	14.46 ± 0.436	14.92 ± 0.436	14.69 ± 0.308
10 % Juncea Meal	14.90 ± 0.436	14.89 ± 0.436	14.89 ± 0.308
20 % Juncea Meal	15.51 ± 0.436	15.27 ± 0.436	15.39 ± 0.308
Enzyme Means	14.96 ± 0.195	14.74 ± 0.195	

Table G.7. Humeri of brown-shell egg laying hens with medullary bone as indicated by bone in the trabecular space

Cage	Meal	Enzyme	Density Values
24	Soybean Meal	No	17.5
81	20 % Canola	No	15.2
91	10 % Juncea	No	74.6
35	20 % Juncea	No	33.1
53	20 % Juncea	No	89.2
100	20 % Juncea	No	60.5
90	Soybean Meal	Yes	14.5
86	20 % Canola	Yes	30.7
96	20 % Canola	Yes	17.1
55	20 % Juncea	Yes	2.5
85	20 % Juncea	Yes	100.8

Table G.8. ANOVA *P*-values for humerus bone quality measurements from brown-shell egg laying hens

	Bone Quality Measurement			
	Weight	Length	Width	Breaking Strength
Source of Variation				
Enzyme	0.5336	0.4933	0.2924	0.1022
Meal	0.9030	0.5751	0.1970	0.8189
Enzyme*Meal	0.3537	0.7108	0.2143	0.3248

Table G.9. Humerus bone quality measurements from brown-shell egg laying hens

Bone Weight (g)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	6.8 ± 0.45	6.1 ± 0.45	6.5 ± 0.32
10 % Canola Meal	7.0 ± 0.45	6.4 ± 0.45	6.7 ± 0.32
20 % Canola Meal	6.5 ± 0.45	7.1 ± 0.45	6.8 ± 0.32
10 % Juncea Meal	6.5 ± 0.45	7.0 ± 0.45	6.7 ± 0.32
20 % Juncea Meal	6.8 ± 0.45	6.1 ± 0.45	6.5 ± 0.32
Enzyme Means	6.7 ± 0.20	6.5 ± 0.20	
Length (cm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	78 ± 0.9	77 ± 0.9	77 ± 0.6
10 % Canola Meal	77 ± 0.9	76 ± 0.9	76 ± 0.6
20 % Canola Meal	77 ± 0.9	78 ± 0.9	77 ± 0.6
10 % Juncea Meal	79 ± 0.9	77 ± 0.9	78 ± 0.6
20 % Juncea Meal	77 ± 0.9	77 ± 0.9	77 ± 0.6
Enzyme Means	77 ± 0.4	77 ± 0.4	
Width (mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	8.1 ± 0.23	8.5 ± 0.23	8.3 ± 0.16
10 % Canola Meal	8.3 ± 0.23	7.7 ± 0.23	8.0 ± 0.16
20 % Canola Meal	8.1 ± 0.23	7.7 ± 0.23	7.9 ± 0.16
10 % Juncea Meal	8.4 ± 0.23	8.5 ± 0.23	8.4 ± 0.16
20 % Juncea Meal	8.2 ± 0.23	8.0 ± 0.23	8.1 ± 0.16
Enzyme Means	8.2 ± 0.10	8.1 ± 0.10	
Breaking Strength (kg Force)			
	Enzyme		
	No	No	Meal Means
Meal			
Soybean Meal	16.12 ± 0.966	13.16 ± 0.966	14.64 ± 0.683
10 % Canola Meal	15.01 ± 0.966	14.05 ± 0.966	14.53 ± 0.683
20 % Canola Meal	16.14 ± 0.966	14.25 ± 0.966	15.19 ± 0.683
10 % Juncea Meal	14.90 ± 0.966	14.79 ± 0.966	14.85 ± 0.683
20 % Juncea Meal	15.14 ± 0.966	15.99 ± 0.966	15.57 ± 0.683
Enzyme Means	15.46 ± 0.432	14.45 ± 0.432	

Appendix H. Data for QCT and quality measurements of tibia of brown-shell egg laying hens.

Table H.1. ANOVA *P*-values for tibia cross-sectional area measurements for brown-shell egg laying hens

	Area		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.7846	0.1794	0.1699
Meal	0.9648	0.5678	0.7887
Enzyme*Meal	0.1905	0.9204	0.5556

Table H.2. Tibia cross-sectional area measurements from brown-shell egg laying hens

Total Area (mm^2)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	59.48 ± 1.953	58.72 ± 1.953	59.10 ± 1.381
10 % Canola Meal	59.31 ± 1.953	58.22 ± 1.953	58.76 ± 1.381
20 % Canola Meal	56.20 ± 1.953	59.23 ± 1.953	57.71 ± 1.381
10 % Juncea Meal	56.04 ± 1.953	60.63 ± 1.953	58.33 ± 1.381
20 % Juncea Meal	60.30 ± 1.953	56.23 ± 1.953	58.27 ± 1.381
Enzyme Means	58.26 ± 0.874	58.60 ± 0.874	
Cortical Area (mm^2)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	25.57 ± 2.107	24.69 ± 2.107	25.16 ± 1.490
10 % Canola Meal	25.24 ± 2.107	22.53 ± 2.107	23.77 ± 1.490
20 % Canola Meal	25.00 ± 2.107	24.33 ± 2.107	24.69 ± 1.490
10 % Juncea Meal	23.70 ± 2.107	23.31 ± 2.107	23.51 ± 1.490
20 % Juncea Meal	26.63 ± 2.107	24.77 ± 2.107	25.65 ± 1.490
Enzyme Means	25.16 ± 0.942	23.90 ± 0.942	
Trabecular Area (mm^2)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	30.52 ± 2.692	30.53 ± 2.692	30.52 ± 1.904
10 % Canola Meal	29.70 ± 2.692	33.37 ± 2.692	31.59 ± 1.904
20 % Canola Meal	28.87 ± 2.692	31.41 ± 2.692	30.16 ± 1.904
10 % Juncea Meal	28.37 ± 2.692	34.39 ± 2.692	31.53 ± 1.904
20 % Juncea Meal	29.58 ± 2.692	27.90 ± 2.692	28.75 ± 1.904
Enzyme Means	29.42 ± 1.204	31.60 ± 1.204	

Table H.3. ANOVA *P*-values for tibia density measurements for brown-shell egg laying hens

	Density		
	Total	Cortical[1]	Trabecular
Source of Variation			
Enzyme	0.2915	0.3216	0.0637
Meal	0.6412	0.9217	0.6170
Enzyme*Meal	0.8473	-	0.8239

[1]Cortical density *P*-values determined using non-parametric Kruskal-Wallis test

Table H.4. Tibia density measurements for brown-shell egg laying hens

Total Density (mg/cm³)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	551.3 ± 23.15	539.1 ± 23.15	545.2 ± 16.37
10 % Canola Meal	554.4 ± 23.15	515.5 ± 23.15	534.9 ± 16.37
20 % Canola Meal	555.6 ± 23.15	546.6 ± 23.15	551.1 ± 16.37
10 % Juncea Meal	536.8 ± 23.15	508.1 ± 23.15	522.4 ± 16.37
20 % Juncea Meal	548.9 ± 23.15	559.9 ± 23.15	554.4 ± 16.37
Enzyme Means	549.4 ± 10.36	533.8 ± 10.36	
Cortical Density (mg/cm³)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	1000.5 ± 20.65	1009.7 ± 20.65	1005.1 ± 14.60
10 % Canola Meal	990.1 ± 20.65	1024.1 ± 20.65	1007.1 ± 14.60
20 % Canola Meal	1003.6 ± 20.65	1016.9 ± 20.65	1010.2 ± 14.60
10 % Juncea Meal	1013.2 ± 20.65	1004.3 ± 20.65	1008.8 ± 14.60
20 % Juncea Meal	969.7 ± 20.65	1017.1 ± 20.65	993.4 ± 14.60
Enzyme Means	995.4 ± 9.23	1014.4 ± 9.23	
Trabecular Density (mg/cm³)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	155.5 ± 20.29	172.4 ± 20.29	164.1 ± 14.35
10 % Canola Meal	161.9 ± 20.29	189.8 ± 20.29	176.4 ± 14.35
20 % Canola Meal	150.1 ± 20.29	180.5 ± 20.29	166.0 ± 14.35
10 % Juncea Meal	126.1 ± 20.29	166.3 ± 20.29	147.6 ± 14.35
20 % Juncea Meal	158.8 ± 20.29	154.8 ± 20.29	156.8 ± 14.35
Enzyme Means	151.0 ± 9.07	173.2 ± 9.07	

Table H.5. ANOVA *P*-values for tibia bone mineral content measurements for brown-shell egg laying hens

	Bone Mineral Content		
	Total	Cortical	Trabecular
Source of Variation			
Enzyme	0.4167	0.3588	0.1528
Meal	0.7220	0.2055	0.7076
Enzyme*Meal	0.6836	0.9067	0.5142

Table H.6. Tibia bone mineral content measurements for brown-shell egg laying hens

Total Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	32.71 ± 1.455	31.66 ± 1.455	32.19 ± 1.029
10 % Canola Meal	32.60 ± 1.455	29.86 ± 1.455	31.23 ± 1.029
20 % Canola Meal	31.05 ± 1.455	32.10 ± 1.455	31.57 ± 1.029
10 % Juncea Meal	30.17 ± 1.455	30.74 ± 1.455	30.46 ± 1.029
20 % Juncea Meal	33.04 ± 1.455	31.46 ± 1.455	32.25 ± 1.029
Enzyme Means	31.92 ± 0.651	31.16 ± 0.651	
Cortical Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	25.47 ± 1.268	25.27 ± 1.186	25.37 ± 0.868
10 % Canola Meal	24.76 ± 1.186	23.22 ± 1.268	23.99 ± 0.868
20 % Canola Meal	25.94 ± 1.186	25.39 ± 1.186	25.66 ± 0.839
10 % Juncea Meal	24.44 ± 1.268	22.90 ± 1.268	23.66 ± 0.897
20 % Juncea Meal	25.83 ± 1.268	26.22 ± 1.186	26.03 ± 0.868
Enzyme Means	25.28 ± 0.560	24.58 ± 0.538	
Trabecular Bone Mineral Content (mg/mm)			
	Enzyme		
	No	Yes	Meal Means
Meal			
Soybean Meal	5.39 ± 0.782	5.51 ± 0.782	5.45 ± 0.553
10 % Canola Meal	5.01 ± 0.782	6.05 ± 0.782	5.55 ± 0.553
20 % Canola Meal	4.89 ± 0.782	5.30 ± 0.782	5.10 ± 0.553
10 % Juncea Meal	3.79 ± 0.782	5.87 ± 0.782	4.94 ± 0.553
20 % Juncea Meal	4.86 ± 0.782	4.45 ± 0.782	4.66 ± 0.553
Enzyme Means	4.82 ± 0.350	5.46 ± 0.350	