**An-Najah National University**

**Faculty of Graduate Studies**

# The Effect of Dietary Inclusion of Distillers Dried Grains with Solubles and Multienzyme Preparation (Avizyme) on Layer Performance and Egg Quality

**By**

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# Dedication

This thesis is dedicated to all the people who never stop believing in me and who along with Allah, have been my ‘footprints in the sand’,

My parents,

My brothers and sisters,

And my friends who have supported me throughout the years of my study. I will always appreciate all they have done.

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I am tempted to individually thank all of my friends, colleagues in the Ministry of Agriculture and University, but as the list might be long, and for fear, I might omit someone, I will simply and genuinely say: Thank you to you all for your love, care and trust.

Eventually, I deeply thank my Family: My parents, brothers and sisters for believing in me and for being proud of me.

I cannot finish without acknowledging how eternally grateful and thankful to Allah.

# الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

**The Effect of Dietary Inclusion of Distillers Dried Grains with solubles and Multienzyme Preparation (Avizyme) on Layer Performance and Egg Quality**

اقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة علمية أو بحث علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

# Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher’s own work, and has not been submitted elsewhere for any other degree or qualification.

| | |
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| **Signature:** | **التوقيع:** |
| **Date:** | **التاريخ:** |

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## List of Abbreviations

| | |
|---|---|
| **AAFCO** | Association of American Feed Control Officials |
| **AME** | Apparent metabolizable energy |
| **AOAC** | Association of Official Analytical Chemists |
| **ANOVA** | Analysis Of Variance between groups |
| **Ca** | Calcium |
| **CF** | Crude Fiber |
| **CP** | Crude Protein |
| **CRD** | Completely randomized design |
| **DCP** | Di-calcium phosphate |
| **DDGS** | Dried Distillers Grain with Solubles |
| **DM** | Dry matter |
| **EE** | Ether Extract |
| **EFSA** | European Food Safety Authority |
| **GE** | Gross Energy |
| **LSD** | Least Significant Difference |
| **ME** | Metabolizable Energy |
| **NRC** | National Research Council |
| **NSP** | Non-starch polysaccharides |
| **P** | Phosphorus |
| **TMEn** | True metabolizable energy |

# List of Appendices

**The Effect of Dietary Inclusion of Distillers Dried Grains with Solubles and Multienzyme Preparation (Avizyme) on Layer Performance and Egg Quality**

**By**

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## Abstract

An experiment was conducted to evaluate the inclusion of distiller dried grain with solubles (DDGS) in commercial layer diets with Avizyme 1505. Three hundred 68 weeks-old Hy-line second cycle layers were distributed in a completely randomized experimental design in 2x4 factorial arrangement, with the variables being (DDGS) substitution for corn and soybean meal at two levels (0 or 15%) and Avizyme 1505 at four levels (0, 100, 150, and 200 gm/ton). Layer performance and egg quality were evaluated. Results showed that DDGS, Avizyme, or their interaction did not significantly affect body weight, egg production, egg weight, and egg mass. The results of this experiment suggest that DDGS level and enzyme supplementation did not significantly influence the body weight, egg production and egg characteristics parameters of second cycle laying hens.Another experiment was conducted to evaluate the effectiveness of commercial layer diets supplemented with varying levels Avizyme 1505 (0,100, 200, 500, 1000 gm/ton). One hundred and fifty 73 weeks-old Hy-line second cycle layers were distributed in a completely randomized design. Layer performance and egg quality were evaluated. The results of the present study indicated that Avizyme at the commercially

recommended level or even higher levels did not significantly affect any of the performance parameters.

# Chapter One

# Introduction

# 1. Introduction:

Agriculture is an essential component of social cultural and national economy in Palestine. The Palestinians were pioneers in the transfer and dissemination of agricultural technologies to several countries in the region and beyond. In addition, it is of particular importance for the Palestinians, as they are a refuge and a source of income and food in times of crisis, also a significant proportion of those who were prevented from working in Israel during the first intifada and the second.

The agricultural sector plays an important role in the national economy in Palestine. Laying hens represent 11.1% of the total livestock production. In the 2010/2011, there was 1.4 million layers in the West Bank according to the last the Agricultural Statistics Survey 2010/2011.

Table eggs are an excellent source of nutrition, including protein, vitamins, and minerals (calcium, iron, phosphorus, zinc and iodine). Eggs are an important source of high quality protein, which is a rich source of the essential amino acid.

Feed ingredients used for poultry, especially soybean meal, are becoming increasingly expensive. Therefore, there is a need to look for means of reducing the cost of diets and for economic alternative protein sources.

Among these means are; alternate feed ingredients (i.e. distillers dried grains with solubles DDGS), and additives (i.e. enzymes, probiotics and prebiotics, organic acids and synthetic amino acids).

The use DDGS depends upon its prices relative to standard feedstuff to save in feed costs. The price of DDGS is expected to be determined relative to prices of standard feedstuff in order to make DDGS profitable for farmers to choose. Otherwise, if DDGS is priced too high therefore it is not chosen by farmers, DDGS have little value in alternative uses.

In the past, available DDGS was from the fermentation of a variety of different grains used by the beverage industry. In the present time, the available DDGS is from corn fermentation in the process of producing ethanol. Since the late 1990s, fuel ethanol production from corn grain has greatly increased, through a fermentation process that is slightly different from those of beverage-alcohol production. As a result, over 98% of the fermentation co-products available today are from fuel-ethanol production using corn grain as a substrate (University of Minnesota, 2008a)

Currently in Palestine, DDGS is available to feed producers. As commercial feed ingredient, prices continue to increase; the use of products such as DDGS to replace portions of the more expensive ingredients will receive more attention. However, local feed producers are reluctant to use DDGS in their diets due to technical and nutritional issues. Economic restraints, the relatively low energy content of DDGS, the bulk density of DDGS meaning that the density of DDGS-containing diets tends to decrease with increasing DDGS content. Pelleting of DDGS, pelleting difficulties comes in part from an increase in the dietary oil content (some of which comes from the DDGS) and in part because DDGS lack starch, which otherwise helps bind the pellets together (Babcock et al., 2008).

Local egg producers prefer corn- soybean based diets for their laying hens. On the other hand, it is believed that addition of commercial enzyme preparations to poultry diets improve ingredients digestibility and nutrient availability because these enzymes counteract the anti-nutritional factors (i.e. non-starch polysaccharides) that are presented in grains.

To date, the use of commercial enzyme preparations containing protease, amylase, and xylanase in corn-soybean based diets did not indicate any success to target anti- nutritional factor that are present in a corn-soybean diet. Consequently, this experiment has been conducted to determine if higher level of Avizyme can target the anti-nutritional factors in corn-soybean diet.

To our knowledge no previous research has been done to investigate the influence of higher (more than the commercial recommended levels) of enzyme preparation.

Therefore, the objectives of this study are to:

1. Evaluate the effects of feeding DDGS on the second cycle laying hens performance;

2. Evaluate the effect of additives (Avizyme 1505) supplementation in diets containing DDGS on second cycle laying hens performance.
3. Evaluate the effect of different levels of enzyme preparation (Avizyme 1505) supplementation in the commercial corn soybean based diets.

# Chapter Two

# Literature Review

# 2. Literature Review:

## 2.1. Poultry Industry in Palestine

The agricultural sector plays an important role in the national economy in Palestine. The value of livestock production (meat, dairy, eggs) in the Palestine during the agricultural year 2007/2008 registered approximately US$ 534.7 million (Palestinian Central Bureau of Statistics, 2007/2008). Laying hens represent 11.1% of the total livestock production. There were 2,695 thousands laying hens in the Palestine including 1,995 thousand birds in the West Bank and 700 thousand birds in the Gaza strip. In the 2010/2011, agricultural year there was 1.4 million layers in the West Bank according to the last the Agricultural Statistics Survey 2010/2011 (Palestinian Central Bureau of Statistics, 2010/2011).

## 2.2. Egg Laying Hens

Almost all commercial egg-laying strains start egg production at five months old (18 weeks of age). A laying hen lays 275-300 eggs per year. Some strains lay about 330 eggs per year. The highly egg productive strains are Isa brown, Babcock, star cross, Hy-line, Lohmann etc. Egg production lasts for 12 to 14 months (the first production cycle). Laying hens undergo an induced molt to allow for a second production cycle, which lasts for an extra 6 months.

Laying hens usually are given a high-energy high protein diets during the first six months of production and then given lower protein diets during the rest of the production cycle. Diets are usually formulated either according to the production guide of the hen's strain or according to the recommendations described by the National Research Council (NRC, 1994).

Egg production of newly matured pullets increases rapidly to a maximum rate of lay (90%) within the first 2 months (around 30 to 32 weeks of age). Egg size also increases at a rapid rate over this period and continues to increase at a slower rate throughout the laying cycle (Leesons and Summer, 1991). Post-peak egg production continually decreases to approximately 50% around the 60 to 70 weeks of age. At this point producers may decide to molt the flock due to economic reasons (e.g., feed cost=market price of eggs), or any other reasons like Ramadan and in summer months when demand for table eggs declines, or when prices of new pullets are too high.

## 2.3. Dried Distillers Grain with Solubles

Poultry Feed constitutes nearly 70 to 80% of the recurring cost of poultry farms, and as such, any reduction in the cost of feed, will go a long way in reducing the cost of production of eggs.  With reduced feed intake and the high egg output, a balance of all nutrients is required for meeting requirements for body maintenance and egg production. Poultry need to get a fixed supply of energy, protein, essential amino acids, minerals, vitamins and, most important, water. Poultry diets are formulated from a mixture of ingredients, including cereal grains, cereal by-products, fats, protein sources, vitamin and mineral supplements, amino acids and feed additives. The increasing cost and decreasing supply of traditional feedstuffs such as soybean and corn are expected to constrain the poultry production.

"Distillers Dried Grains with Soluble (DDGS) is the product obtained after the removal of ethyl alcohol by distillation from the yeast fermentation of a grain or a grain mixture by condensing and drying at least ¾ of the solids of the resultant whole stillage and drying it by methods employed in the grain distilling industry". (Official publication of the Association of American Feed Control Officials AAFCO (2006-2007) definition). As a result, the non-fermentable components of this process, which are rich in essential nutrients such as protein, fat, fiber, vitamins and minerals, are recovered in a highly concentrated form (approximately 3 fold) as distillers dried grains with solubles (NRC, 1994; Weigel *et al.*, 1997; AAFCO 2002).

Corn distillers dried grains with solubles (DDGS) is a byproduct obtained from the milling of corn, and possibly other grains, for ethanol production. Increased attention on ethanol production in the United States and Worldwide will certainly continue to increase the production of DDGS radically (Shurson, 2003).

During corn fermentation, microbes turn the starch component into alcohol, with carbon dioxide being released as a byproduct. The remaining portion (1/3 of the original corn is the protein, fat, fiber and ash which are not fermented by the microbes. It has been reported that DDGS has a nutrient profile mid-way between that of corn and soybean meal. Therefore, it looks promising for inclusion in poultry feeds and in replacing some of the corn and soybean meal in poultry diets (Shurson, 2003).

Several studies (Shurson, 2003) reported a nutrient profile for a light colored DDGS as follow: 2820 ME kcal/kg, 27.5% crude protein, 10% fat, 5.5% crud fiber, 4.25% ash and a dry matter of 89%.

Recently, DDGS is becoming available and it is derived entirely from corn and is dried under less extreme thermal conditions. Despite this, the nutritional profile of a given DDGS sample can be highly variable depending in processing and drying temperatures (Batal and Dale, 2006; and Fastinger *et al.*, 2006).

There has been several researches conducted on the use of corn DDGS in diets of layers, confirming that it is an excellent partial replacement for corn, soybean meal and supports acceptable layer performance and egg quality.

Matterson *et al.* (1966) reported that DDGS could be added to layers diets at levels of 10 to 20% (inclusion level) without causing negative effects on egg production even with no synthetic lysine supplementation.

Jensen *et al.* (1974) reported that interior egg quality was improved by supplementing layers diet with DDGS at 5-20% inclusion level of diet even as a source of one-third of the protein supply; however, there was not a consistent response.

Lumpkins *et al.* (2005) evaluated the use of high quality corn DDGS in layers diets. These authors fed hy-line w-36 laying hens high energy (True Metabolizable Energy TME) (2871 kcal TMEn/kg) and low energy (2805 kcal TMEn/kg) diets, with and without 15% DDGS from 22 to 42 weeks of age. These researchers concluded that DDGS is a very acceptable feed ingredient in layer diets and the maximal dietary inclusion level of DDGS should be 10 to 12% in high-energy commercial diets, but lower dietary inclusion rates may be necessary in lower energy diets.

Roberson *et al.* (2005) conducted two experiments where diets containing 0, 5, 10, or 15% DDGS were fed to laying hens to study the effect of DDGS levels in egg production parameters and yolk color. In the first experiment, DDGS (golden colored corn) was added to diets fed from 48 to 56 weeks of age and then another source of DDGS (brown colored) was added to diets from 58 to 67 weeks of age. Egg production measurements were not affected at most ages. However, as dietary levels of DDGS increased, there was a corresponding decrease in egg production, egg weight, egg mass and specific gravity. Egg yolk color increased as dietary level of DDGS increased throughout the experiment. In the second experiment, similar responses were observed. These authors concluded that feeding layer diets containing up to 15% DDGS did not affect egg production, but the variable results suggest that a level less than 15% DDGS should be used.

Cheon *et al.* (2008) studied in a 10 week experiment, the effect of 0, 10, 15 and 20% inclusion rates of DDGS (light colored) in a layer diets on laying performance, egg quality and yolk fatty acid composition. These authors concluded that light colored DDGS could be used at levels up to 20% in layer diets without any negative effect on laying hen performance. Brunet and Ingram (2013), reported similar results. It is generally recommend that layer diets may contain DDGS with inclusion rates varying from 5-20%. On the other hand feeding up to 12% of corn DDGS to laying hens had no effect on egg weight, feed intake, egg yolk color, and exterior or interior egg quality (Jung and Batal, 2009).

Loar *et al.* (2010) fed a second cycle Bovans White laying hens a commercial diet formulated to contain 0, 8, 16, 24, or 32% DDGS for a period of 15 week, to study the effect of varying levels of DDGS on layer performance, egg characteristics, and consumer acceptability. These authors concluded that feeding up to 32% DDGS in diets to second-cycle layers had no detrimental effects on production. In addition, increasing DDGS in the diet led to a slightly darker egg yolk.

Masa’deh *et al.* (2011) conducted two layer feeding trials for hens from 24 to 46 weeks (phase 1) and from 47 to 76 weeks (phase 2). These authors fed diets containing 0, 5, 10, 15, 20 or 25% DDGS. Diets were formulated to be isocaloric (2775 and 2816 kcal/kg of ME) and isonitrogenous (16.5 and 16.0% crud protein) in phase 1 and 2, respectively. These authors showed that adding up to 25%, DDGS in layer diets had no negative effect on feed intake, egg production, Haugh unit, or specific gravity and that egg yolk color was improved at higher inclusion rates. These authors concluded that including DDGS at levels greater than 15% during the first phase decreased egg weight but this was not the case during the second phase.

Recently, it has been found that if corn DDGS exceeded 15% it will causes a slight decrease in production and deterioration in egg quality and performance of laying hens (Niemiec *et al.*, 2013). These authors fed laying hens a mixture containing 15 or 20% corn DDGS for 18 weeks. These authors found that the inclusion of DDGS in the feed mixture had no negative effect on egg weight and feed intake. They concluded that 15% addition of corn DDGS to feed mixture for layer hen diets is advisable.

The incorporation of DDGS in poultry diets is limited mainly by the presence of large amounts of insoluble non-starch polysaccharides (NSP), lower nutrient digestibility and wide nutrient variation between grain sources and production batches (Barekatain *et al.*, 2012).

Several studies have indicated that corn and soybean meal are incompletely digested by poultry (Pack and Bedford, 1997, and Marsman *et al.*, 1997). For instance, several studies, with broilers, indicated improvement in energy and protein digestibilities of corn-soy broiler diets due to supplementation of α- galactosidase enzyme (Pack and Bedford, 1997; Kidd *et al.*, 2001). Scheideler *et al.* (2005) reported improvement in some apparent nutrient retention: (nitrogen, protein and calcium) by hens given diets supplemented with xylanase, protease and amylase (Avizyme 1500). Other researchers indicated that corn DDGS should be included in layers diet at less than 15.45% of total dietary level, supplemented with Avizyme 1500 (a commercial enzyme) in order to improve egg productive performance (Ghazalah *et al.*,2011).

Others concluded that addition of enzymes to DDGS containing diets, improved the utilization of DDGS even at 15% or 20% (Shalash *et al.*, 2010). Recently, Deniz *et al*, (2013) reported that, using an enzyme cocktail affect anti-nutritional factors in corn DDGS and may improve the nutritive value of corn DDGS when given to laying hens.

## 2.4. Exogenous Enzyme Preparation in Poultry Nutrition

Feeding enzymes to poultry has been considered by many nutritionists as one of the major advances in the last few decades. Animals, especially monogastrics, cannot produce the necessary enzymes to digest antinutritional factors that are present in most plants. These enzymes usually come from microorganism that are selected and grown under controlled conditions (Wallis, 1996).

Bacteria (*Bacillus subtilis, Busillus lentus, Basillus amyloliquifaciens* and Bacillus *stearothermophils*), fungus (*Triochoderma longibrachiatum,* *Asperigillus oryzae* and *Asperigillus niger*) and Yeast (*S. cerevisiae*) are microorganisms involved in production of enzymes. These enzymes are essential for metabolic process, and they were used in the preparation of food and beverage industry (Khattak *et al.*, 2006).

Enzyme supplementation in the feed plays an important role in increasing the availability of nutrients and alleviating the adverse effect of anti-nutritional factors that are present in the feed components (*Khusheeba* and Maqsood, 2013). The goals of adding the enzymes to animal rations are to increase the digestibility and to remove the anti-nutritional factors. The most common anti-nutritive factors are the non-starch polysaccharides (NSPs). Exogenous enzymes hydrolyze non-starch polysaccharides (NSPs) (Buchanan *et al.*, 2007). These enzymes might be used in animals diets such as for poultry since birds do not produce enzymes for the hydrolysis of such anti-nutritional factor presented in the cell wall of the grains. Also the presence of pentosans in wheat, oligosaccharides in soybean and phytates in every vegetable ingredient limit energy, protein and phosphorus digestibility of diets (Schang and Azcona, 2003).

Many researchers studied the effect of using enzyme supplementation in layer rations. There are many types of enzyme used (β-glucanases, Xylanases, β-galactosidases, Phytases, Proteases, Lipases, and Amylases). These enzymes are used in the feed industry for poultry to neutralize the effect of anti-nutritive factors, or as feed additive as phytases to liberate P from plant feeds, protease for protein digestion, lipase for lipid digestion, β-galactosidases for neutralizing anti-nutritive factors in non-cereal feedstuff and amylase in the digestion of starch (Khattak *et al*., 2006).

The benefits of using enzymes is to enhance digestion and absorption of nutrients like fat and protein, and to improve the apparent metabolizable energy (AME) value have been well studies (Campbell *et al*., 1989; Jansson *et al*.,1990; Wang *et al*., 2005). Khattak *et al*. (2006) have shown that enzymes are significant instrument for better use of poultry feeds. Others reported that pure enzyme supplementation increased the protein metabolizability, NSPs digestibilities, apparent metabolizable energy (AME) and retention of calcium, phosphorus, phytate phosphorus and nitrogen in laying hens, which helps in better utilization of alternate feed ingredients (Ramesh and Chandrasekaran, 2011).

Yoruk *et al*., (2006) conducted an experiment to evaluate the effects of multi-enzyme supplementation on laying performance, metabolic profile and egg quality of peak producing hens. Lohman layers, received one of three corn-soybean meal based diets supplemented with multi-enzymes (0, 1, or 2 g/kg) from 30 to 46 weeks of age. At the end of the experiment, these authors found that supplementation of a multi-enzyme to a corn-soybean diet did not negatively affect on body weight, feed consumption and egg production.

Flores-Cervantes *et al*. (2011) studied the effect of using an enzyme blend in a sorghum-soymeal-based ration (protein 17.5% and ME 2900 kcal/kg) on performance. These authors used two lines: Hy-line and

Bovans white, at eighteen weeks of production, these authors concluded that multi-enzyme mixture has a minor effect on performance.

Malekian *et al.* (2013) studied the effect of multi-enzyme supplementation for 42 weeks old broiler breeders fed a corn-soy bean based diet. The experiment lasted for 10 weeks. These authors concluded that addition of multi-enzyme preparation slightly increased egg production and egg mass.

Wu et al., (2005) studied the effect of β-mannanase on performance of commercial Leghorns fed corn-soybean meal based diets. In this experiment, three diets were formulated. The metabolizable energy content for diet 1(high-energy diet) was 2,951 kcal/kg, which was 120 kcal/kg higher than diet two (low-energy diet supplemented with β-mannanase) and diet three (low-energy dietwithout β-mannanase). The trial lasted for 12 week. These authors found that the addition of β-mannanase significantly increased average egg production and egg mass of hens fed the low-energy diet from week 5 to 8. There were no significant differences in feed intake, egg specific gravity, egg weight, mortality, body weight, and body weight variability among the three dietary treatments.

Costa *et al*., (2008) conducted an experiment with 72-week-old second-cycle laying hens. These authors fed commercial diets and diets containing commercial exogenous enzymes. These authors concluded that enzyme supplementation was efficient in increasing egg production in second cycle laying hens.

In a recent review (Slominski, 2011) pointed out that using of the currently available commercial enzymes in poultry diets, especially corn-soybean ones, have been unsuccessful. The use of a blend of dietary enzymes has not been investigated in corn-soybean diets supplemented with DDGS.

Several studies were conducted to study the use of different types of Avizyme in layer diets, (Sinurat et al., 2012) studied the efficacy of avizyme 1500 for improving performance of laying hens, these authors conducted Two treatments, the control diet (diet based on corn – soybean meal) (C) and C + 1000 g Avizyme/tonne diet were tested. Each diet was fed to 80 birds from 20 to 72 weeks of age. They concluded that the addition of Avizyme 1500 to the feed reduced feed intake, the egg production, egg size and egg mass however were not significantly affected by the Avizyme supplementation. Egg quality (HU, yolk colour score, yolk weight and shell thickness) was not significantly affected by Avizyme supplementation.

In our study we used Avizyme 1505 which is a complex of (1500 endo-1,4-beta-xylanase (xylanase), 20000 subtilisin and 2000 alpha-amylase U/g) ( EFSA ,2011). Many field researches have been carried out to evaluate the efficacy of Avizyme in diets of egg laying hens. These studies concluded that Avizyme can be safety added to diets of hens to improve egg production and egg mass (EFSA, 2011)

# Chapter Three

# Materials and Methods

# 3. Materials and methods

## 3.1.Experiment 1

### 3.1.1Experimental Design

A 5 weeks experiment was carried out using 300 second cycle Hy-line laying hens to evaluate the effectiveness of diets containing two levels of DDGS (0 and 15%) supplemented with exogenous enzyme preparations (Avizyme 1505).

Avizyme 1505 is a commercial microbial multi-enzyme preparation that has xylanase, protease and amylase activity. Four levels of Avizyme 1505 (0, 100, 150, and 200 gm/ton) were used.

These factorial combinations (DDGS and Avizyme 1505) resulted in eight dietary treatments. Thus, treatments were factorially arranged and consisted of two DDGS levels (0 and 15%) and four levels of Avizyme (0, 100, 150, and 200 gm/ton).

The 300 hens were used throughout the experimental period. Hens were housed individually in open-sided house in the facility (double-deck cages) of the Faculty of Agriculture Farm (Khadouri). Each 10 cages were considered as an experimental unit, thus each treatment was replicated three times. Hens were managed according to common practices recommend by Hy-line management guide (Hyline, 2011).

### 3.1.2. Dietary Treatments

Prior to the initiation of the experiment, hens were given a commercial laying diet that meet the nutrient requirements of laying hens (NRC, 1994) for two weeks.

The profile of the commercial diet: protein 17%, fat 5%, fiber 5%, moisture 13%, ash 13%, Ca 4%, P 0.55%, NaCl 0.35%, and Mn 80 gm/ton .(The manufacturer does not usually provide the composition of the commercial diet).

Then hens were then fed the dietary treatments (table 1) throughout the experimental period.

Two corn-soybean based rations were formulated: one contained no DDGS (0% DDGS) and the other contained 15% DDGS. Each patch was subdivided into four rations and each was supplemented with one level of (0, 100, 150, and 200 gm/ton) of Avizyme 1505.

The experimental diets were formulated to meet National Research Commercial (NRC, 1994) nutrient requirements of laying hens. Diets were formulated to be iso-nitrogenous and to have similar proportion of the essential amino acids, calcium and phosphorous.

Dietary ingredient were purchased from a local feed mill, and rations were formulated in the Farm of the Faculty of Agriculture. Formulated diets were iso-caloric, iso-nitrogenous and were fed in mash form.

Hens were randomly assigned to each dietary treatment and were given a fixed daily amount of feed (110 gm per hen per day). Hens were fed the experimental diets for 5 weeks beginning at 69 weeks of age.

The composition and the chemical analysis of the experimental diets are shown in table 1.

**Table (1): The composition and the calculated analysis of the experimental diets.**

| Ingredient[1]/ Quantity/Kg | 0 (%) DDGS | 15 (%) DDGS |
|---|---|---|
| Yellow Corn | 583.4 | 511.5 |
| SBM | 260.7 | 186.0 |
| Oil | 29 | 29 |
| DDGS[2] | -- | 150 |
| DL-methionine | 0.999 | 0.434 |
| Salt | 2.997 | 3.015 |
| limestone | 110.9 | 110.5 |
| DCP | 10.9 | 8.5 |
| Vitamin-mineral premix[3] | 0.999 | 1.005 |
| Total | 999.9 | 999.7 |
| The price of one Ton( NIS) | 1733 | 1625 |
| **Calculated analysis** | | |
| Dry matter | 87.8 | 88.2 |
| Crude protein | 17.3 | 17.4 |
| Fat | 5.2 | 5.1 |
| Fiber | 2.3 | 2.7 |
| Ca | 4.4 | 4.4 |
| P | 0.5 | 0.5 |
| Ash | 3.1 | 2.9 |
| Lysine | 0.9 | 0.9 |
| Methionine | 0.4 | 0.4 |
| Cysteine | 0.3 | 0.3 |
| Tryptophane | 0.2 | 0.2 |
| Threonione | 0.7 | 0.7 |
| ME (kcal/kg) | 2811 | 2833 |

[1] Each of experimental diets was supplemented with graded levels of Avizyme as follows: 0, 100, 150, and 200 gm/ton resulting in 8 dietary treatments.

[2] Chemical analysis of DDGS: CP 27.9%, fat 9.4%, fiber 6.47%, calcium 0.05%, phosphorus 0.82%

[3] Vitamin-mineral premix contains/5kg : 7MIU vitamin A, 2MIU vitamin D3, 10000IU vitamin E, 2g vitamin K3, 1g thiamine, 4g riboflavin, 10g niacin, 5g pantothenic acid, 0.75g pyridoxine,0.25g folic acid, 0.008g vitamin B12, 0.04g biotin, 200g choline chloride, 125g monox, 80g manganese, 50g zinc, 1.2g iodine, 0.2g cobalt, 5g copper, 20g iron, 0.2g selenium, 2500g sodium chloride, 1000g sodium sulfate.

### 3.1.3. Birds and Management.

A total of 300-second cycle Hy-line laying hens were randomly distributed into eight experimental treatments each with three replicates. Initial weight was obtained for each bird at the beginning of the experiment. The birds were housed individually in open-sided house in the facility (double-deck cages) at the Faculty of Agriculture Farm (Khadouri).

Predetermined daily allowance of feed was served manually, and the hens had access to water from cup drinkers connected to municipality water pipes.

### 3.1.4. Parameters Measured.

Egg production was recorded daily for 5 weeks beginning at 69 weeks of age and continuing to 73 weeks of age. Egg weight, yolk weight, albumen weight and shell weight were obtained from eggs collected during the last two days of each week for each replicate. A digital egg scale (Breville electronic scale) was used to weight eggs. These same eggs were then carefully broken and albumen, yolk, and shell were separated and weighed. Eggshell thickness was measured by manual micrometer, before measuring the eggshell thickness, the eggshells have to be cleaned from faeces, yolk remains etc, then from the equator of the cleaned egg three little pieces are taken for the measurement. Egg out-put (mass) was calculated by multiplying the average egg weight by the total number of eggs produced by hens in each replicate.

### 3.1.5. Statistical Analysis.

Data for egg production, Egg weight, yolk weight, albumen weight and shell weight were analyzed using the general linear model of SAS (SAS Institute, 2000) subjected to the analysis of variance (ANOVA) as a factorial arrangement. LSD test (Least Significant Difference) was applied for mean comparisons, with DDGS level and enzyme level as main effects along with the interaction of these two effects. Replicate mean was the experimental unit for performance. Differences at $P < 0.05$ were considered significant. The model for CRD (completely randomized design) with a factorial arrangement is:

$$Y_{ijk} = u + DDGS_i + Enz_j + (DDGS*Enz)_{ij} + e_{ijk}$$

Where $DDGS_i$ is the effect of levels of the factor DDGS, and the $Enz_j$ is the effect of levels of the factor Enzyme. $(DDGS*Enz)_{ij}$ is the effect of the interaction of levels of level i of the factor DDGS with level j of the factor Enzyme , and $e_{ijk}$ is the random error.

## 3.2. Experiment 2:

### 3.2.1. Experimental Design.

A 2 weeks experiment was carried out using 150 second cycle Hy-line laying hens to evaluate the effectiveness of diets supplemented with different levels of exogenous enzyme preparations (0, 100, 200,500, 1000 gm/ton). The hens, 73 weeks of age, were used throughout the experimental period, which lasted for 2 weeks. Hens were housed individually in open-sided house in the facility (double-deck cages) of the Faculty of Agriculture Farm (Khadouri). Each 10 cage was considered as an experimental unit, thus each treatment was replicated three times. Hens were managed according to common practices recommended by Hy-line management guide (Hyline, 2011).

### 3.2.2. Dietary Treatments.

Prior to the initiation of the experiment, hens were given a commercial laying diet as in experiment 1. Then hens were fed the dietary treatments (Table 2), One dietary treatment was formulated according to the commercial standards whereas the other treatments were supplemented with different levels of exogenous enzyme preparations (Avizyme); this resulted in five dietary treatments.The commercial corn soybean meal based diet served as the control (treatment 1); treatment 2, 3, 4 and 5 supplemented with different levels of exogenous enzyme preparations (Avizyme 1505) (0, 100, 200, 500 and 1000 gm/ton) respectively. Dietary ingredient purchased from a local feed mill, and formulated in the experimental farm of the Faculty of Agriculture. All diets formulated to be iso-caloric, iso-nitrogenous and fed in mash form. Diets were formulated to meet nutrient recommendation (NRC, 1994) for layers.

Hens were given a daily allowance (110 gm) of feed. Calculated analysis of the dietary treatments is shown in (table 2).

**Table (2): The Ingredients Compostion and Calculated analysis of the experimental diets.**

| Ingredient[1] | Quantity(Kg) |
|---|---|
| Yellow Corn | 583.4 |
| SBM | 260.7 |
| Fat/Oil | 29 |
| DL-methionine | 0.999 |
| Salt | 2.997 |
| limestone | 110.9 |
| DCP | 10.9 |
| Vitamin-mineral premix[2] | 0.999 |
| Total | 999.9 |
| **Calculated analysis** | |
| Ingredient | (%) |
| Dry matter | 87.8 |
| Crude protein | 17.3 |
| Fat | 5.2 |
| Fiber | 2.3 |
| Ca | 4.4 |
| P | 0.5 |
| Ash | 3.1 |
| Lysine | 0.9 |
| Methionine | 0.4 |
| Cysteine | 0.3 |
| Tryptophane | 0.2 |
| Threonione | 0.7 |
| ME (kcal/kg) | 2851.5 |

[1] Each of experimental diets were supplemented with graded levels of Avizyme as follows: 0, 100, 200, 500 and 1000 gm/ton resulting in five dietary treatments.

[2] Vitamin-mineral premix contains/5kg: 7MIU vitamin A, 2MIU vitamin D3, 10000IU vitamin E, 2g vitamin K3, 1g thiamine, 4g riboflavin, 10g niacin, 5g pantothenic acid, 0.75g pyridoxine,0.25g folic acid, 0.008g vitamin B12, 0.04g biotin, 200g choline chloride, 125g monox, 80g manganese, 50g zinc, 1.2g iodine, 0.2g cobalt, 5g copper, 20g iron, 0.2g selenium, 2500g sodium chloride, 1000g sodium sulfate.

### 3.2.3. Birds and Management.

A total of 150-second cycle Hy-line laying hens (73 weeks of age) were randomly distributed into five experimental treatments each with three replicates, each replicate contained 10 birds. Initial body weight was obtained for each bird at the beginning of the experiment and the birds were housed individually in the facility (double-deck cages) at the Faculty of Agriculture Farm (Khadouri).Hens were given a fixed (110gm/day) amount of feed and provided free access to water.

### 3.2.4. Parameters Measured.

Body weight was recorded for individual hens at the beginning and at the termination of the experiment. Egg production was recorded daily. Egg weight, yolk weight, albumen weight, and eggshell weight were measured for 2 eggs produced in the last two days of each week for each replicate by using a digital egg scale (Breville electronic scale). Before measuring the eggshell thickness, the eggshells have to be cleaned from faeces, yolk remains etc, then from the equator of the cleaned egg three little pieces are taken for the measurement and was measured using manual micrometer to obtain eggshell thickness. Egg out-put (mass) was calculated by multiplying the average egg weight by number of eggs produced by hens in each replicate.

### 3.2.5. Statistical Analysis.

Data for all variables measured were subjected to one-way analysis of variance using the general linear model GLM for mean comparisons, with enzyme level as main effect. Replicate means was the experimental unit for performance. Differences at $P \leq 0.05$ were considered significant. The model CRD is:

$$Yij=u+Enzi+eij$$

Where **Enzi** is the fixed effect of the treatments and the eij is the random error.

# Chapter Four

# Results

# 4. Results.

## 4.1. Experiment 1.

### 4.1.1. Body Weight Characteristic.

Initial body weight, final body weight and body weight difference of second-cycle laying hens receiving supplemental Avizyme (0, 100, 150, and 200 gm/ton) in diets containing 0 or 15% DDGS are shown in (Table 3).

No significant differences in body weight gain was observed among hens receiving diets with or without DDGS (Table 3); however, hens receiving diets with no added DDGS and no enzyme lost weight, but weight loss was not significant compared to hens receiving the other diets.

**Table 3: Body weight difference of second cycle hens fed diet with 0 or 15% DDGS supplemented with different levels of Avizyme.**

| Body weight characteristic | Levels of DDGS (%) | Level of Avizyme (gm/ton) | | | |
|---|---|---|---|---|---|
| | | 0 | 100 | 150 | 200 |
| Initial body Weight (kg) | 0 | 1.713$^{abc}$±0.050 | 1.587$^{c}$±0.050 | 1.753$^{ab}$±0.050 | 1.647$^{abc}$±0.050 |
| | 15 | 1.720$^{abc}$±0.050 | 1.773$^{a}$±0.050 | 1.630$^{abc}$±0.050 | 1.627$^{abc}$±0.050 |
| Final body (kg) | 0 | 1.670$^{bc}$±0.054 | 1.660$^{c}$±0.054 | 1.890$^{a}$±0.054 | 1.673$^{bc}$±0.045 |
| Weight (kg) | 15 | 1.830$^{ab}$±0.045 | 1.823$^{ab}$±0.045 | 1.723$^{bc}$±0.045 | 1.767$^{abc}$±0.045 |
| Body weight differences[1](Kg) | 0 | -0.043$^{c}$±0.053 | 0.073$^{abc}$±0.053 | 0.136$^{ab}$±0.053 | 0.027$^{abc}$±0.053 |
| | 15 | 0.110$^{ab}$±0.053 | 0.050$^{abc}$±0.053 | 0.093$^{abc}$±0.053 | 0.140$^{a}$±0.053 |

[1]Based on differences of initial body weight at 69 weeks and final body weight at 73 weeks.
$^{abc}$ Means with the same letter are not significantly different.

### 4.1.2. Production Performance.

Number of eggs, average egg weight and egg mass (egg output) of second-cycle laying hens receiving supplemental Avizyme (0, 100, 150, and 200 gm/ton) in diets containing 0 or 15% DDGS is shown in (Table 4). Egg production did not differ significantly (P>0.05) for hens fed 0 DDGS or 15% DDGS (Table 4). In addition, levels of enzyme did not significantly affect egg production. Hens receiving diets with 15% DDGS supplemented with 150 gm/ton enzyme laid slightly more eggs than hens receiving the other diets.

Eggs from hens receiving the control diet were not significantly different from that receiving the diet supplemented with 15% DDGS (Table 4). In addition, there were no significant differences in the egg weight from hens fed different levels of Avizyme.

**Table (4): Egg production of second cycle hens fed diets, with 0 or 15% DDGS supplemented with different levels of Avizyme.**

| Performance Characteristics | DDGS Levels % | Avizyme levels (gm/ton) | | | |
|---|---|---|---|---|---|
| | | **0** | **100** | **150** | **200** |
| Number of eggs | 0 | 156.3[a]±28.4 | 161.7[a]±28.4 | 145.0[a]±28.4 | 115.3[a]±28.4 |
| | 15 | 138.0[a]±28.4 | 157.0[a]±28.4 | 173.0[a]±28.4 | 147.3[a]±28.4 |
| Average Egg weight (gm) | 0 | 77.8[a]±1.7 | 75.8[a]±1.7 | 76.2[a]±1.7 | 76.9[a]±1.7 |
| | 15 | 77.9[a]±1.7 | 74.6[a]±1.7 | 78.4[a]±1.7 | 76.9[a]±1.7 |
| Egg Mass(kg)[1] | 0 | 12.09[a]±2.21 | 12.27[a]±2.21 | 11.12[a]±2.21 | 8.91[a]±2.21 |
| | 15 | 10.74[a]±2.21 | 11.69[a]±2.21 | 13.61[a]±2.21 | 11.33[a]±2.21 |

[1]Eggmass=Egg weight x Total number of egg through 5 weeks of experiment period.

[a] Means with the same letter are not significantly different.

### 4.1.1.3. Egg Characteristics.

Egg quality parameters (egg shell weight, egg shell thickness, albumen weight, and yolk weight) of hens receiving diet with 15% DDGS were not significantly different than that of hens receiving the control diet (Table 5).There was no difference in the egg parameters between hens receiving diets with 0, 100, 150, or 200 gm/ton Avizyme.

**Table (5): Egg quality parameters of second cycle hens fed diets, with 0 or 15% DDGS supplemented with different levels of Avizyme.**

| Egg Characteristics | Levels of DDGS (%) | Levels of Avizyme (gm/ton) | | | |
|---|---|---|---|---|---|
| | | 0 | 100 | 150 | 200 |
| Egg shell Weight (gm) | 0 | $11^{ab}\pm0.30$ | $10.7^{ab}\pm0.30$ | $10.2^{b}\pm0.30$ | $10.5^{ab}\pm0.30$ |
| | 15 | $11.1^{a}\pm0.30$ | $10.6^{ab}\pm0.30$ | $10.8^{ab}\pm0.30$ | $10.5^{ab}\pm0.30$ |
| Egg shell Thickness(mm) | 0 | $0.380^{a}\pm0.012$ | $0.367^{a}\pm0.012$ | $0.370^{a}\pm0.012$ | $0.363^{a}\pm0.012$ |
| | 15 | $0.376^{a}\pm0.012$ | $0.372^{a}\pm0.012$ | $0.365^{a}\pm0.012$ | $0.370^{a}\pm0.012$ |
| Albumen Weight(gm) | 0 | $45.7^{a}\pm1.53$ | $45.3^{a}\pm1.53$ | $46.7^{a}\pm1.53$ | $47.5^{a}\pm1.53$ |
| | 15 | $47.2^{a}\pm1.53$ | $44.2^{a}\pm1.53$ | $48.2^{a}\pm1.53$ | $46.3^{a}\pm1.53$ |
| Yolk Weight (gm) | 0 | $19.13^{a}\pm0.34$ | $18.97^{a}\pm0.34$ | $18.50^{a}\pm0.34$ | $18.77^{a}\pm0.34$ |
| | 15 | $18.40^{a}\pm0.34$ | $18.07^{a}\pm0.34$ | $18.33^{a}\pm0.34$ | $18.73^{a}\pm0.34$ |

[ab] Means with the same letter are not significantly different.

## 4.2. Experiment 2.

### 4.2.1. Body Weight.

Initial body weight, final body weight and body weight difference of second-cycle laying hens receiving supplemental Avizyme (0, 100, 200, 500 and 1000 gm/ton) in diets is shown in (Table 6). At the end of the 2 weeks experimental period, all hens gained weight except for those on diet with 100gm and 500gm added enzyme supplementation.

Although initial body weight of hens given 1000gm/ton enzyme, there were no significant difference (P>0.05) among dietary treatments with regard to body weight of hens when using the different levels of Avizyme. Hens fed diet containing 500gm/ton had the high weight loss by the end of the experiment.

**Table (6): Body weight difference of second cycle hens fed diets supplemented with 0, 100, 200, 500 and 1000gm/ton Avizyme.**

| **Body weight characteristic** | **Levels of Enzyme (gm)** | | | | |
|---|---|---|---|---|---|
| | **0** | **100** | **200** | **500** | **1000** |
| Initial weight (kg) | 1.72[ab] ±0.055 | 1.84[ab] ±0.055 | 1.71[ab] ±0.055 | 1.86 [a] ±0.055 | 1.67[b] ±0.055 |
| Final weight (kg) | 1.85 [a] ±0.043 | 1.80 [a] ±0.043 | 1.85 [a] ±0.043 | 1.79 [a] ±0.043 | 1.73 [a] ±0.043 |
| Body weight Difference(gm) | 0.131[ab] ±0.063 | -0.036[ab] ±0.063 | 0.141 [a] ±0.063 | -0.065[b]±0.063 | 0.055[ab] ±0.063 |

[ab] Means with the same letter are not significantly different.

### 4.2.2. Production Performance.

The production results obtained in the present study indicate that a significant difference were observed in egg production and cumulative egg production when hens were fed the dietary treatments with different levels of Avizyme over the  2 weeks of experiment. Moreover, that was clearly observed when hens were fed the diets containing 200gm/ton Avizyme level in comparison with the other treatments. However, supplemental Avizyme (0, 100, 200, 500 and 1000 gm/ton) in diets did not affect egg weight; data is shown in (Table 7).  In addition, egg mass was significantly different depending on the number of eggs produced using the experimental diets during the experiment period.

**Table (7): Number of eggs, average egg weight, and egg mass of second cycle hens receiving 0, 100, 200, 500 and 1000gm/ton Avizyme in diets.**

| Performance Characteristics | Levels of Avizyme (gm/ton) | | | | |
|---|---|---|---|---|---|
| | **0** | **100** | **200** | **500** | **1000** |
| Number of eggs | 74.7[ab] ±6.22 | 64.7[bc]±6.22 | 52.3[c] ±6.22 | 67.7 [abc]±6.22 | 86.7 [a]±6.22 |
| Average egg Weight (gm) | 76.0 [a] ±1.38 | 75.3 [a] ±1.38 | 76.3 [a] ±1.38 | 74.0 [a] ±1.38 | 75.0 [a] ±1.38 |
| Egg mass [1](kg) | 5.68[ab] ±0.47 | 4.86[bc] ±0.47 | 3.99 [c] ±0.47 | 5.02[abc] ±0.47 | 6.50 [a] ±0.47 |

[1]Eggmass=Egg weight X Total number of egg through 5 weeks of experiment period.

[abc]Means with the same letter are not significantly different.

### 4.1.2.3. Egg Characteristics.

Egg quality parameters (eggshell weight, eggshell thickness, albumen weight, and yolk weight) of second-cycle laying hens receiving supplemental Avizyme (0, 100, 200, 500 and 1000 gm/ton) in diets are shown (Table 8). It can be seen that eggshell weight, albumen weight, and yolk weight were not affected significantly (P>0.05) by the experimental diets supplemented with Avizyme (0, 100, 200, 500, and 1000gm/ton) during the experimental period of 2 weeks.

**Table (8): Egg quality of second cycle hens receiving 0, 100, 200,500 and 1000gm/ton Avizyme in diets.**

| Egg Characteristics | Levels of Avizyme (gm/ton) | | | | |
|---|---|---|---|---|---|
| | 0 | 100 | 200 | 500 | 1000 |
| Egg shell weight (gm) | 10.3[a]±0.15 | 10.0[a]±0.15 | 10.0[a]±0.15 | 10.0[a]±0.15 | 10.0[a]±0.15 |
| Eggshell thickness(mm) | 0.329[a] ±0.01 | 0.339[a] ±0.01 | 0.337[a]±0.01 | 0.347[a]±0.01 | 0.326[a]±0.01 |
| Albumen weight(gm) | 46.7[a]±1.35 | 47.0[a]±1.35 | 48.7[a]±1.35 | 45.7[a]±1.35 | 46.7[a]±1.35 |
| Yolk weight (gm) | 19.7[a]±0.39 | 19.3[a]±0.39 | 19.3[a]±0.39 | 20.0[a]±0.39 | 19.3[a]±0.39 |

[a] Means with the same letter are not significantly different.

# Chapter Five

# Discussion

# 5. Discussion:

## 5.1. Experiment One.

### 5.1.1. Body Weight Characteristic.

It has been revealed by the results of the present study that hens body weights were not significantly affected by level of DDGS, enzyme or by interactions of DDGS and enzyme levels. Indeed the research result is fully in accordance with the results reported by previous studies (Lumpkins *et al*., 2005; Jung and Batal, 2009; Shalash *et al*., 2010; Masa'deh *et al.*, 2011; Niemiec *et al.*, 2013).These authors have confirmed that DDGS at 15% inclusion rate had no negative consequences in hens body weight.

In the present study during the post molt production period, body weight of hens receiving the control diet (with no added DDGS and enzyme preparation) had numerically lower body weight compared to weight of hens fed the other diets. These results are in disagreement with those reported by Masa'deh *et al.* (2012) who reported that body weight gain was lower for hens receiving up to 15% DDGS. These authors fed DDGS at 0, 5, 10, 15, 20 or 25% DDGS to first cycle laying pullets. However, these authors did not use enzyme supplementation. Therefore, the discrepancy between our results and theirs can be explained by the fact that hens during the first production cycle are supposed to gain weight but hens in our study were in the second laying cycle. Lumpkins *et al*. (2005)

and Shurson *et al.* (2003) also reported that feeding DDGS had no effect on hen body weight when fed at 15% or 10% levels, respectively. Ghazalah et al. (2011), reported that Avizyme supplementation had no significant effect on body weight when added with DDGS supplemented diet.

**5.1.2. Production performance:**

No differences in egg weight and hen day egg production, egg mass were observed among dietary treatments. Our data were similar to Lumpkins *et al.* (2005) and Roberson *et al.* (2005). These authors conducted studies with laying hens incorporating up to 15% DDGS with no negative effect on egg production. Egg production results indicate no negative effect of DDGS (at 15% level) on hen performance. Furthermore, the egg production was not influenced by using different levels of Enzyme. Our research results are in agreement with previous studies conducted by Lumpkins *et al.* (2005).

These authors conducted an experiment on laying hens by using 15% of DDGS. The revealed results pointed out that there had been negative effects on egg production even when the hens fed diets of high energy (2871 kcal TMEn/kg) and low energy (2805 kcal TMEn/kg). The level of energy used in our study was in between the level of energy used by the above authors (2833 kcal/kg). This is mainly attributed to the prevailed cold temperature during our research period conducted in Tulkarem between December and January 2013.

Others (Cheon *et al*., 2008; Jung and Batal, 2009; Masa'deh *et al*., 2011; Niemiec *et al*., 2013) investigated the effect of corn DDGS added to feed mixtures for laying hens, and found that it had no effect on laying performance even if it was used at 20-25%.

Loar *et al*. (2010) fed a second cycle laying hens with a commercial diet formulated to contain 0, 8, 16, 24, or 32% DDGS, and concluded that feeding up to 32% DDGS in diets to second-cycle layers had no detrimental effects on production. Their results agreed with the results of the current study.

The lack of significant differences in the mean egg weigh in the present study is consistent with the results of Loar *et al*. (2010) and Ghazalah *et al*. (2011), but inconsistent with results reported by (Masa'deh *et al.*, 2011). The latest authors found a reduction in egg weight when DDGS level increased in the feed mixture. In contrast, Ghazalah *et al.* (2011) reported that Avizyme addition improved egg production and egg mass for DDGS inclusion level at 25 and 50%. The data reported by these authors were similar to those reported in our study.

Egg weight and egg mass were not affected by DDGS treatment or enzyme supplementation. There were no significant interaction effects between level of DDGS and enzyme inclusion for any of the production parameters. Our data are similar to those of (Lumpkins *et al.*, 2005) and

(Roberson *et al.*, 2005). Both authors have conducted experiments with laying hens incorporating up to 15% DDGS with no negative effects on the following parameters: egg production, egg weigh, and egg mass. However, (Roberson *et al.*, 2005) reported a linear decrease in egg production (52-53 week of age), egg weigh (63 week of age), and egg mass (51 week of age) during certain periods and when the level of DDGS increased.

Shalash *et al*. (2010) showed that increasing DDGS to 15 or 20% in laying hen diets significantly decreased egg hen day production, egg weight and egg mass. Lower levels of DDGS did not affect these parameters. Enzyme addition to DDGS diets give a hand in improving the utilization of DDGS levels even with the high levels 15 or 20% (Shalash *et al*., 2010). This is evident in our study in that Avizyme addition prevented negative effect in egg production.

In recent study of (Deniz *et al.*, 2013) who reported that, feeding up to 15% medium-quality corn DDGS with or without enzyme cocktail supplementation had no negative effects on performance parameters (i.e., percentage laying rate, egg weight, feed intake and feed conversion). Moreover, there was no interaction between the inclusion levels of corn DDGS and the supplementation of enzyme cocktail on performance. The results in our study were in agreement with those reported by the above-mentioned authors.

**5.1.3. Egg Characteristics.**

In the current study, egg quality parameters (i.e., eggshell weight, eggshell thickness, albumen weight, and yolk weight) of hens received diet at 15% DDGS with or without the addition of Avizyme (0, 100, 150, or 200 gm/ton) were not significantly different from those received the control diet. In addition, there were no interaction between DDGS level and the enzyme supplementation.

Lumpkins *et al*. (2005) concluded that feeding 15% DDGS to laying hens had no effect on exterior or interior egg quality, which is in agreement with our results in the current study. Jung and Batal, (2009) agreed with our results when they found that feeding hens up to 12% DDGS had no effect on the exterior or the interior egg quality.

Cheon *et al*. (2008) showed that no differences in weigh, strength, and color of eggshell were detected when feeding (0, 10, 15, and 20% DDGS). Other authors (Roberson *et al*., 2005; Loar *et al*., 2010) did not demonstrate the effect of DDGS on the quality of eggshell. Whereas (Ghazalah *et al*., 2011) showed a decrease of shell thickness when increasing DDGS ratio in the diet. Niemiec *et al*., (2013) reported that with DDGS addition exceeding 15% deterioration in egg quality was observed.

Results in the current study was also in agreement with those obtained in the recent study of (Deniz *et al.*, 2013). These authors concluded that feeding up to 15% DDGS with or without enzyme supplementation had no negative effects on exterior (eggshell thickness and shell breaking strength) and interior (Haugh units and egg yolk color) egg quality parameters in the study. Moreover, the interaction between DDGS level and the supplementation of enzyme cocktail had no effect on egg quality. The results of this experiment concluded that DDGS level and enzyme supplementation did not significantly influence the body weight, egg production and egg characteristics parameters.

### 5.2. Experiment 2.

It has been reported (Slominski, 2011) that the use of commercial non-specific enzyme preparations containing protease, amylase, and xylanase to target the two main nutrients of a corn-soybean diet and its non-starch polysaccharides components has been unsuccessful.

Jalal *et al.*(2007), used energy- and protein- deficient as negative control corn-soybean meal diets supplemented with different enzyme preparations for laying hens and observed no significant differences for egg production, feed conversion ratio, and egg weight. The result of the present study also indicated that Avizyme at the commercially recommended levels or even higher levels did not significantly affect any of the performance measures.

#### 5.2.1. Body weight.

Based on the obtained results, it has been concluded that hen body weigh was not affected by applying different levels of Avizyme (0, 100, 200, 500 and 1000 gm/ton).

All the experimental groups gained weigh except those in the group supplemented with (100 and 500 gm/ton) Avizyme, this might be attributed to the environmental condition or to the changes occurred while changing diets components at the beginning of the experiment, but it was not significant. Our results are in agreement with the results obtained by (Wu et al., 2005) who studied the effect of β-Mannanase (unique enzyme-based) in Corn-Soy diets on commercial leghorns in second-cycle hens and found that no significant difference in body weight when adding the β-Mannanase in the diet. Our results were similar to the results obtained by (Yoruk *et al*., 2006), who used a supplementation of a multi-enzyme (0, 1 or 2gm/kg) to a corn-soybean diet for Lohman hens. These authors found no effect on body weight.

**5.2.2. Production performance.**

Our results are in agreement with the results obtained by (Costa *et al*., 2008; Malekian *et al.*, 2013) in which enzyme supplementation was efficient in increasing egg production and egg mass. The difference between the control diet and diets supplemented with Avizyme was significant ($P<0.05$) for egg mass. The egg mass was determined by two components, egg weight and egg production. The similar trend of egg mass and egg production implies that variability in egg mass was mainly due to differences in egg production. Wu et al., (2005) obtained similar results, and (Yoruk *et al*., 2006) showed that no changes in egg production. In contrast to our results (Flores-Cervantes *et al*., 2011) there had been no differences (P>0.05) among enzymatic treatments on egg yield, and egg mass the same as in the study conducted by (Sinurat et al., 2012) in which they concluded that egg production and egg mass were not affected by the Avizyme supplementation.

In the contrast to our results, (Wu *et al*., 2005) reported that diets supplemented with β-mannanase, a part of the multi-enzyme Rovabio, had significantly increased egg weight in some weeks only.

**4.2.2.3. Egg Characteristics.**

The present study showed that it was clear that egg quality measures (eggshell weight, eggshell thickness, albumen weight, and yolk weight) of second-cycle laying hens were not affected significantly (P>0.05) when diets were supplemented with Avizyme (0, 100, 200, 500, and 1000gm/ton). In contrast, Malekian *et al*. (2013) observed significant improvements in eggshell quality in broiler breeder. Our results are in agreement with those obtained by Yoruk *et al*. (2006); Flores-Cervantes et al. (2011). In contrast, Yoruk *et al*. (2006) found that the effect of the multi-enzyme supplementation on egg quality parameters was lacking. Similar to our results (Sinurat et al., 2012) found that egg quality (yolk weight and eggshell thickness) was not significantly affected by the Avizyme supplementation.

## Conclusions:

The results of the current study indicated that DDGS level and enzyme supplementation did not significantly influence the body weight, egg production and egg characteristics parameters.

The result of the present study also indicated that Avizyme at the commercially recommended levels or even higher levels did not significantly affect any of the performance measures.

## Recommendations:

It is recommended that imported DDGS can be safely used (15% inclusion rate) in egg laying hens diets and that Avizyme at the commercially recommended levels.

# References

Association of American Feed Control Officials (AAFCO), 2006-2007. DDGS Definition.

AAFCO (2002) Official publication of the Association of American Feed Control Officials**, Inc.Oxford, IN**.

Babcock B. A., D. J. Hayes, J. D. Lawrence, 2008. Using Distillers Grains in the U.S. and International Livestock and Poultry Industries. **Handbook**.

Barekatain, M. R., M.Chock, C.Antipatis and P.A. Iji, 2012. Use of Protease and xylanase in broiler diets containing distillers dried grains with solubles. **Aust.Poult. Sci. Symp. 65-68**

Batal, A.B., and N.M. Dale, 2006. True Metabolizable Energy and Amino Acid Digestibility of Distillers Dried Grains with Solubles. **J.App. Poult.Res.15:89-93.**

Berry W.D, Brake J, 1985. Comparison of parameters associated with molt induced by fasting zinc and low dietary sodium in caged layers. **Poult. Sci. 64: 2027–2036.**

Bourdillon, A., B. Carre, L. Conan, J. Duperray, G. Huyghebaert, B. Leclercq, M. Lessire, J. McNab, and J. Wiseman. 1990. European reference method for the in vivo determination of metabolizable energy with adult cockerels: Reproducibility, effect of food intake and comparison with individual laboratory methods**. Brit. Poult. Sci. 31:557-565.**

Buchanan, N.P., L.B. Kimbler and A.S. Paarsons, 2007. The Effect of Non-starch Polysaccharide enzyme addition and Dietary energy restriction on Performance and Carcass Quality of Organic Broiler Chickens**. J.Appl. Poult. Res., 16:1-12.**

Brunet L.R. and D.R. Ingram, 2013. The Effects of Distiller's Dried Grains with Solubles on Egg Production and Yolk Color. **Poster**.

Campbell, G.L., B.G. Rossnagel., H.L. Classen and P.A. Thacker., 1989. Genotypic and Environmental differences in extract Viscosity of Barley and their Relationship to its Nutritive Value for Broiler Chickens. **Animal Feed Sci. and Tec. 226:221-230**.

Cheon, Y. J., H. L. Lee, M. H. Shin, A. Jang, S.K. Lee, J. H. Lee, B. D. Lee and C. K. Son,2008. Effects off corn Distiller's Dried Grains with Solubles on Production and Egg Quality in Laying Hens. **Asian-Aust. J. Anim. Sci. 21(9): 1318-1323.**

Costa, F. G. P., C. F. S. Oliveira, C.C. Goulart, D.F.Figueiredo and R.C.L. Neto, 2008. Use of Exogenous Enzymes on Laying Hens Feeding During the Second Production Cycle**. Inte. Jor.poult. Sci. 7(4): 333-338.**

Deniz, G. , H. Gencoglu, S.S. Gezen, I.I. Turkmen, A. Orman, C. Kara, 2013.Effects of feeding corn distiller's dried grains with solubles with and without enzyme cocktail supplementation to laying hens on performance, egg quality, selected manure parameters, and feed cost. **Livestock Science, 152(2): 174-181**.

European Food Safety Authority (EFSA), 2011. Scientific Opinion on the safety and efficacy of Avizyme 1505 (endo-1,4-beta-xylanase, subtilisin and alpha amylase) as feed additive for laying hens. **EFSA Journal 2011; 9(1):1949.**

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

Flores-Cervantes, Sergio, Juan C. Casillas-Franco, and José-Rogelio Orozco-Hernández, 2011. Effect of a Multi-enzymatic Mix in a Sorghum-soybean Meal-based Ration on Hen Performance. **Italian J. of Animal Sci. 10:e25**

Ghazalah, A.A.,M.o. Abd-Elsamee, and Eman S. Moustafa, 2011. Use of Distillers Dried Grain with Solubles (DDGS) as Replacement for Soybean Meal in Laying Hen Diets. **Int. Jour.of Poult. Sci. 10(7):505-513.**

Hy-line International, 2011. West Des Moines. IA.

Jalal, M., S. Scheideler, and E. Pierson. 2007. Strain response of laying hens to varying dietary energy levels with and without Avizyme supplementation. **J. App. Poult. Res. 16(3): 289-295**.

Jansson, L., K. Elwinger, B. Engstrom, O. Fossum and B. Telgof, 1990. Test of the Efficacy of Virginiamycin and dietary Enzyme Supplementation against Necrotic Enteritis Disease in Broilers.

Proceedings, **8th European Poultry Conference, Barcelona, Spain. Pp. 556-559.**

Jensen, L. S., L. Falen, and C.H. Chang, 1974. Effect of Distillers Grains with Solubles on Reproduction and Liver Fat Accumulation in Laying Hens**. Poult. Sci. 53:586-592**.

Jung, B. and A. Batal, 2009. The nutrient Digestibility of High-Protein Corn Distiller Dried Grain and the Effect of Feeding Various Levels on the Performance of Laying Hens. **J. Appl. Poult. Res. 18:741-751.**

Khattak, F. M., T. N. Pasha, Z. Hayat and A. Mahmud, 2006. Enzyme in Poultry Nutrition**. J. Anim. Pl. Sci. 16(1-2).**

Kidd, M.T., G.W. Morgan, Jr., C.J. Price, P.A. Welch, and E.A. Fontana, 2001.Energy supplementation to corn and soybean meal diets for broilers**. J. Appl. Poult. Res. 10:65-70.**

Khusheeba, M., and S. Maqsood, 2013. A review on role of exogenous enzyme supplementation in poultry production. **Emir. J. Food Agric. 25 (1): 66-80.**

Loar II, R.E.,M.W. Schilling, C.D. Mc Daniel, C.D. Coufal, S.F. Rogers, Karges and A. Corzo, 2010. Effect of Dietary Inclusion level of distillers dried grains with solubles on layer performance, egg characteristics and consumer acceptability. J. Appl. Poult. Res. 19:30-37.

Lumpkins, B., A. Batal, and N. Dale, 2005.Use of distiller dried grains plus solubles in laying hens diets**. J. Appl. Poult. Res. 14:25-31.**

Malekian, Gh., A.K. Zamani Moghaddam and F. Khajali, 2013. Effect of Using Enzyme Complex on Productivity and Hatchability of Broiler Breeders Fed a Corn-Soybean Meal Diet**. Poult. Sci. J., 1 (1): 36-45**

Marsman, G.H. Gruppen, A.Van de Poel, R. Kwakkel, M.Verstegen, and A. Voragen, 1997. The effect of thermal processing and enzyme treatments of soybean meal on growth performance, ileal nutrient digestibility, and characteristics in broiler chicks**. Poult. Sci.76:864-872.**

Masa'deh, M.K., S.E. Purdum and K.J. Hanford, 2011. Distillers dried grain with solubles in Laying Hen Diets. **Poult. Sci. 90:1960-1966**.

Matterson, L.D., J. Tlustohowicz, and E. P. Singsen, 1966. Corn Distillers Dried Grains with Solubles in Rations for High-Producing Hens. **Poult. Sci. 45:147-151**.

NRC, 1994. Nutrient Requirements of Poultry. **9th rev. ed. Natl. Acad. Press,Washington, DC.**

Niemiec, J., J. Riedel, T. Szulc and M. Stepinska, 2013. Feeding Corn Distillers Grains with Solubles (DDGS) and its Effect on Egg Quality and Performance of Laying Hens. **Ann. Anim. Sci. 13(1):97-107.**

Pack, M., and M.Bedford, 1997. Feed enzymes for corn and soybean broiler diets. **World Poult. Sci. 13:87-93.**

Palestinian Central Bureau of Statistics, **2010/2011.**

Ramesh, J. and D. Chandrasekaran, 2011.Effect of Pure Enzyme Supplementation on Digestibility and Metabolizability of Nutrients in Laying Hens. **Tamilnadu J. Veterinary and Animal Sci. 7(2)79-87.**

Roberson, K.D., J.L. Kalbfleisch, W. Pan, and R.A. Charbeneau, 2005. Effect of corn distillers' dried grain with solubles at various levels on performance of laying hens and yolk color. **Int. Poult. Sci. 4:44-51.**

SAS Institute, 2000. SAS User's Guide: Statistics - **SAS Institute Cary. NC.**

Shalash, S.M.M. ; El-Wafa, S. A. ; Hassan, R.A. ; Ramadan, N. A. ; Mohamed, M.S. ; El-Gabry, H. E., 2010. Evaluation of Distillers Dried Grains with Solubles as Feed Ingredient in Laying Hen Diets. **Int. J. Poult. Sci., 9 (6): 537-545**

Schang, M.J. and J.O. Azcona, 2003. Natural Enzyme Applications to Optimize Animal Performance. In: Alltech's Annual Symposium, 19, 2003, Lexington. Proceedings … Lexington: **Alltech, pp: 54-67.**

Scheideler,S.E., M.M. Beck, A. Abudabos and C.L. Wyatt, 2005. Multiple-enzyme (Avizyme) supplementation of corn-based layer diets. **J. Appl. Poult. Res. 14:77-86.**

Shurson,J., 2003. The Value and Use of Distiller Dried Grain with Solubles (DDGS) in Livestock and Poultry Rations**. http://www.ddgs.umn.edu/.**

Slominski, B.A., 2011. Recent advances in research on enzymes for poultry diets. **Poult. Sci. 90: 2013-2023.**

Sinurat, A.P, P.P. Ketaren , A.J. Cowieson and M.H.L. Bento, 2012. The efficacy of Avizyme 1500 for improving performance of laying hens. **JITV 17(3): 221-228.**

University of Minnesota, Department of Animal Science. 2008a. Overview. Distillers Grains By-Products in Livestock and Poultry Feeds **Website.http://www.ddgs.umn.edu/overview**.

Wallis, I., 1996. Enzyme in Poultry Nutrition. Technical Note, SAC. West Mains road, Edinburgh.

Wang, Z. R., S.Y. Qiao, W. Q. Lu and D.F. Li, 2005. Effect of Enzyme supplementation on Performance, Nutrient Digestibility, Gastrointestinal Morphology, and Volatile Fatty Acid Profile in the Hindgut of Broilers Fed Wheat-Based Diet**. Poult. Sci. 84: 875-881**.

Weigel, J.C., Loy, D. and Kilmer, L.,1997. Feed Co- Products of the Dry Corn Milling Process.Renewable Fuels Association and National

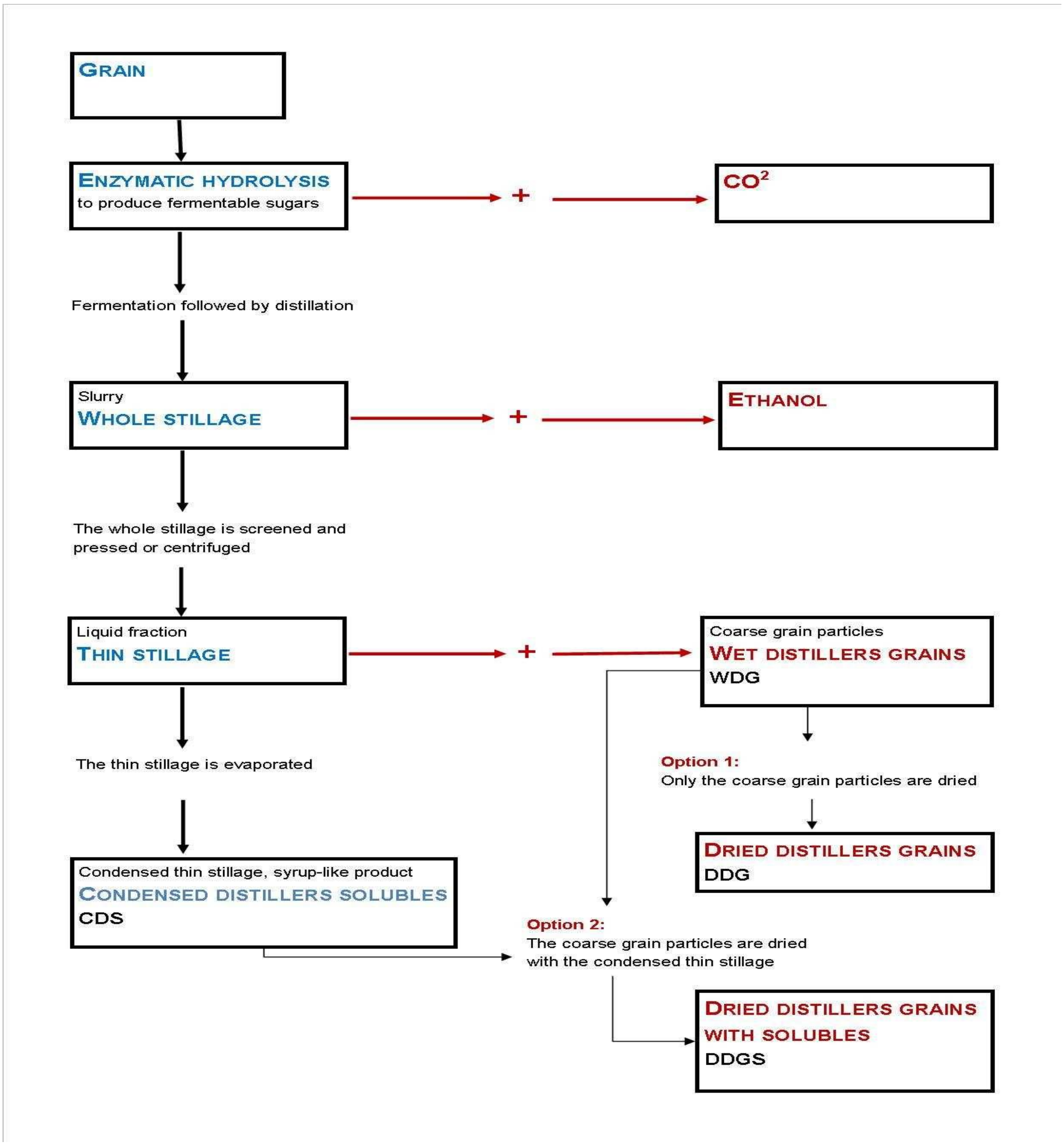
Corn Growers Association.**Washington, D.C. and St. Louis,Missouri, USA.**

Wu, G., M. M. Bryant, R. A. Voitle, and D. A. Roland, 2005. Effects of β-Mannanase in Corn-Soy Diets on Commercial Leghorns in Second-Cycle Hens. **Poult. Sci. 84:894–897**

Yoruk, M.A, Gul M, Hayirli A, Karaoglu M, 2006. Multi-Enzyme Supplementation to Peak Producing Hens Fed Corn-Soybean Meal Based Diets. **Inte. J. of Poult. Sci. 5(4): 374-380**

# Annex (1): Flow chart of the DDGS-process

Adapted from:




Hans Grinsted Jensen, Andreas H. Björnsson, Kim Martin Lind, 2013. IFRO Report: By-products from ethanol production the forgotten part of the equation (Possibilities and challenges).

# Annex (2)

## Statisitcal Analysis (Experiment One)

### analysis of 2*4 factorial

| Obs | DDGS | Enz | inwt | fwt | negg | aveggwt | Eshwt | eshT | Albwt | yolkwt | Bwdif | Eggmass |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 1.80 | 1.63 | 132 | 81.0 | 11.0 | 0.38575 | 47.0 | 19.7 | -0.17 | 10692.0 |
| 2 | 0 | 0 | 1.67 | 1.74 | 141 | 78.5 | 11.0 | 0.38550 | 47.0 | 19.0 | 0.07 | 11068.5 |
| 3 | 0 | 0 | 1.67 | 1.64 | 196 | 74.0 | 11.0 | 0.36850 | 43.0 | 18.7 | -0.03 | 14504.0 |
| 4 | 0 | 12.5 | 1.59 | 1.80 | 97 | 75.0 | 10.0 | 0.35100 | 44.7 | 19.3 | 0.21 | 7275.0 |
| 5 | 0 | 12.5 | 1.60 | 1.62 | 121 | 76.5 | 11.0 | 0.36350 | 45.0 | 19.3 | 0.02 | 9256.5 |
| 6 | 0 | 12.5 | 1.57 | 1.56 | 267 | 76.0 | 11.0 | 0.38750 | 46.0 | 18.3 | -0.01 | 20292.0 |
| 7 | 0 | 18.8 | 1.89 | 2.02 | 97 | 77.5 | 9.5 | 0.35900 | 47.5 | 18.7 | 0.13 | 7517.5 |
| 8 | 0 | 18.8 | 1.71 | 1.78 | 122 | 72.5 | 10.5 | 0.38450 | 44.0 | 17.5 | 0.07 | 8845.0 |
| 9 | 0 | 18.8 | 1.66 | 1.87 | 216 | 78.7 | 10.5 | 0.36600 | 48.0 | 19.3 | 0.21 | 16999.2 |
| 10 | 0 | 25 | 1.72 | 1.77 | 95 | 77.0 | 10.0 | 0.35200 | 48.0 | 19.3 | 0.05 | 7315.0 |
| 11 | 0 | 25 | 1.59 | 1.66 | 110 | 72.7 | 10.7 | 0.37100 | 42.5 | 18.0 | 0.07 | 7997.0 |
| 12 | 0 | 25 | 1.63 | 1.59 | 141 | 81.0 | 10.7 | 0.36450 | 52.0 | 19.0 | -0.04 | 11421.0 |
| 13 | 15 | 0 | 1.70 | 1.84 | 126 | 77.0 | 10.7 | 0.38575 | 47.0 | 18.7 | 0.14 | 9702.0 |
| 14 | 15 | 0 | 1.69 | 1.83 | 154 | 76.0 | 11.0 | 0.35200 | 45.5 | 18.0 | 0.14 | 11704.0 |
| 15 | 15 | 0 | 1.77 | 1.82 | 134 | 80.7 | 11.5 | 0.39100 | 49.0 | 18.5 | 0.05 | 10813.8 |
| 16 | 15 | 12.5 | 1.88 | 1.94 | 130 | 73.5 | 11.0 | 0.39250 | 42.5 | 19.0 | 0.06 | 9555.0 |
| 17 | 15 | 12.5 | 1.69 | 1.82 | 139 | 76.5 | 10.0 | 0.33525 | 47.0 | 17.7 | 0.13 | 10633.5 |
| 18 | 15 | 12.5 | 1.75 | 1.71 | 202 | 73.7 | 10.7 | 0.38750 | 43.0 | 17.5 | -0.04 | 14887.4 |
| 19 | 15 | 18.8 | 1.76 | 1.74 | 135 | 75.5 | 11.0 | 0.34175 | 45.5 | 18.5 | -0.02 | 10192.5 |
| 20 | 15 | 18.8 | 1.47 | 1.64 | 149 | 80.0 | 11.0 | 0.39875 | 49.5 | 18.5 | 0.17 | 11920.0 |
| 21 | 15 | 18.8 | 1.66 | 1.79 | 235 | 79.7 | 10.5 | 0.35325 | 49.5 | 18.0 | 0.13 | 18729.5 |
| 22 | 15 | 25 | 1.57 | 1.77 | 155 | 73.0 | 11.0 | 0.36350 | 43.0 | 18.5 | 0.20 | 11315.0 |
| 23 | 15 | 25 | 1.67 | 1.86 | 123 | 78.0 | 9.5 | 0.35750 | 48.0 | 19.0 | 0.19 | 9594.0 |
| 24 | 15 | 25 | 1.64 | 1.67 | 164 | 79.7 | 11.0 | 0.38775 | 48.0 | 18.7 | 0.03 | 13070.8 |

Analysis of 2*4 factorial
The GLM Procedure
Class Level Information

| Class | Levels | Values |
|---|---|---|
| DDGS | 2 | 0 15 |
| Enz | 4 | 0 12.5 18.8 25 |

Number of observations 24

## Initial Body weight

Analysis of 2*4 factorial

The GLM Procedure

Dependent Variable: inwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 0.09586250 | 0.01369464 | 1.81 | 0.1546 |
| Error | 16 | 0.12120000 | 0.00757500 | | |
| Corrected Total | 23 | 0.21706250 | | | |

| R-Square | Coeff Var | Root MSE | inwt Mean |
|---|---|---|---|
| 0.441635 | 5.176772 | 0.087034 | 1.681250 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.00093750 | 0.00093750 | 0.12 | 0.7296 |
| Enz | 3 | 0.02011250 | 0.00670417 | 0.89 | 0.4698 |
| DDGS*Enz | 3 | 0.07481250 | 0.02493750 | 3.29 | 0.0478 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.00093750 | 0.00093750 | 0.12 | 0.7296 |
| Enz | 3 | 0.02011250 | 0.00670417 | 0.89 | 0.4698 |

| | | | | | |
|---|---|---|---|---|---|
| DDGS*Enz | 3 | 0.07481250 | 0.02493750 | 3.29 | 0.0478 |

| Parameter | | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | | 1.626666667 B | 0.05024938 | 32.37 | <.0001 |
| DDGS | 0 | 0.020000000 B | 0.07106335 | 0.28 | 0.7820 |
| DDGS | 15 | 0.000000000 B | . | . | . |
| Enz | 0 | 0.093333333 B | 0.07106335 | 1.31 | 0.2076 |
| Enz | 12.5 | 0.146666667 B | 0.07106335 | 2.06 | 0.0556 |
| Enz | 18.8 | 0.003333333 B | 0.07106335 | 0.05 | 0.9632 |
| Enz | 25 | 0.000000000 B | . | . | . |
| DDGS*Enz | 0 0 | -0.026666667 B | 0.10049876 | -0.27 | 0.7941 |
| DDGS*Enz | 0 12.5 | -0.206666667 B | 0.10049876 | -2.06 | 0.0564 |
| DDGS*Enz | 0 18.8 | 0.103333333 B | 0.10049876 | 1.03 | 0.3191 |
| DDGS*Enz | 0 25 | 0.000000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.000000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.000000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.000000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.000000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

# Analysis of 2*4 factorial
## The GLM Procedure
## Least Squares Means

| DDGS | inwt LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 1.67500000 | 0.02512469 | <.0001 | 0.7296 |
| 15 | 1.68750000 | 0.02512469 | <.0001 | |

| Enz | inwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 1.71666667 | 0.03553168 | <.0001 | 1 |
| 12.5 | 1.68000000 | 0.03553168 | <.0001 | 2 |
| 18.8 | 1.69166667 | 0.03553168 | <.0001 | 3 |
| 25 | 1.63666667 | 0.03553168 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: inwt

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.4761 | 0.6256 | 0.1309 |
| 2 | 0.4761 | | 0.8193 | 0.4012 |
| 3 | 0.6256 | 0.8193 | | 0.2899 |
| 4 | 0.1309 | 0.4012 | 0.2899 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | inwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 1.71333333 | 0.05024938 | <.0001 | 1 |
| 0 | 12.5 | 1.58666667 | 0.05024938 | <.0001 | 2 |
| 0 | 18.8 | 1.75333333 | 0.05024938 | <.0001 | 3 |
| 0 | 25 | 1.64666667 | 0.05024938 | <.0001 | 4 |
| 15 | 0 | 1.72000000 | 0.05024938 | <.0001 | 5 |
| 15 | 12.5 | 1.77333333 | 0.05024938 | <.0001 | 6 |
| 15 | 18.8 | 1.63000000 | 0.05024938 | <.0001 | 7 |
| 15 | 25 | 1.62666667 | 0.05024938 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: inwt

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.0937 | 0.5813 | 0.3621 | 0.9264 | 0.4109 | 0.2581 | 0.2403 |
| 2 | 0.0937 | | 0.0322 | 0.4109 | 0.0790 | 0.0183 | 0.5506 | 0.5813 |
| 3 | 0.5813 | 0.0322 | | 0.1528 | 0.6454 | 0.7820 | 0.1019 | 0.0937 |
| 4 | 0.3621 | 0.4109 | 0.1528 | | 0.3174 | 0.0937 | 0.8175 | 0.7820 |
| 5 | 0.9264 | 0.0790 | 0.6454 | 0.3174 | | 0.4638 | 0.2235 | 0.2076 |
| 6 | 0.4109 | 0.0183 | 0.7820 | 0.0937 | 0.4638 | | 0.0608 | 0.0556 |
| 7 | 0.2581 | 0.5506 | 0.1019 | 0.8175 | 0.2235 | 0.0608 | | 0.9632 |
| 8 | 0.2403 | 0.5813 | 0.0937 | 0.7820 | 0.2076 | 0.0556 | 0.9632 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for inwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.007575 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.0753 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 1.68750 | 12 | 15 |
| A | | | |
| A | 1.67500 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for inwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.007575 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.1065 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 1.71667 | 6 | 0 |
| A | | | |
| A | 1.69167 | 6 | 18.8 |
| A | | | |
| A | 1.68000 | 6 | 12.5 |
| A | | | |
| A | 1.63667 | 6 | 25 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of DDGS | Level of Enz | N | -------------inwt------------ Mean | Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 1.71333333 | 0.07505553 |
| 0 | 12.5 | 3 | 1.58666667 | 0.01527525 |
| 0 | 18.8 | 3 | 1.75333333 | 0.12096832 |
| 0 | 25 | 3 | 1.64666667 | 0.06658328 |
| 15 | 0 | 3 | 1.72000000 | 0.04358899 |
| 15 | 12.5 | 3 | 1.77333333 | 0.09712535 |
| 15 | 18.8 | 3 | 1.63000000 | 0.14730920 |
| 15 | 25 | 3 | 1.62666667 | 0.05131601 |

## Final weight

Analysis of 2*4 factorial
The GLM Procedure

Dependent Variable: fwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 0.15772917 | 0.02253274 | 2.56 | 0.0567 |

| | | | |
|---|---|---|---|
| Error | 16 | 0.14086667 | 0.00880417 |
| Corrected Total | 23 | 0.29859583 | |

| R-Square | Coeff Var | Root MSE | fwt Mean |
|---|---|---|---|
| 0.528236 | 5.347738 | 0.093831 | 1.754583 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.02343750 | 0.02343750 | 2.66 | 0.1223 |
| Enz | 3 | 0.02457917 | 0.00819306 | 0.93 | 0.4487 |
| DDGS*Enz | 3 | 0.10971250 | 0.03657083 | 4.15 | 0.0235 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.02343750 | 0.02343750 | 2.66 | 0.1223 |
| Enz | 3 | 0.02457917 | 0.00819306 | 0.93 | 0.4487 |
| DDGS*Enz | 3 | 0.10971250 | 0.03657083 | 4.15 | 0.0235 |

| Parameter | | Estimate | | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| Intercept | | 1.766666667 | B | 0.05417308 | 32.61 | <.0001 |
| DDGS | 0 | -0.093333333 | B | 0.07661230 | -1.22 | 0.2408 |
| DDGS | 15 | 0.000000000 | B | . | . | . |
| Enz | 0 | 0.063333333 | B | 0.07661230 | 0.83 | 0.4206 |
| Enz | 12.5 | 0.056666667 | B | 0.07661230 | 0.74 | 0.4702 |
| Enz | 18.8 | -0.043333333 | B | 0.07661230 | -0.57 | 0.5795 |
| Enz | 25 | 0.000000000 | B | . | . | . |
| DDGS*Enz | 0 0 | -0.066666667 | B | 0.10834615 | -0.62 | 0.5470 |
| DDGS*Enz | 0 12.5 | -0.070000000 | B | 0.10834615 | -0.65 | 0.5274 |
| DDGS*Enz | 0 18.8 | 0.260000000 | B | 0.10834615 | 2.40 | 0.0289 |
| DDGS*Enz | 0 25 | 0.000000000 | B | . | . | . |
| DDGS*Enz | 15 0 | 0.000000000 | B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.000000000 | B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.000000000 | B | . | . | . |
| DDGS*Enz | 15 25 | 0.000000000 | B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial

The GLM Procedure
Least Squares Means

| DDGS | fwt LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 1.72333333 | 0.02708654 | <.0001 | 0.1223 |
| 15 | 1.78583333 | 0.02708654 | <.0001 | |

| Enz | fwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 1.75000000 | 0.03830615 | <.0001 | 1 |
| 12.5 | 1.74166667 | 0.03830615 | <.0001 | 2 |
| 18.8 | 1.80666667 | 0.03830615 | <.0001 | 3 |
| 25 | 1.72000000 | 0.03830615 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: fwt

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.8797 | 0.3111 | 0.5874 |
| 2 | 0.8797 | | 0.2477 | 0.6945 |
| 3 | 0.3111 | 0.2477 | | 0.1292 |
| 4 | 0.5874 | 0.6945 | 0.1292 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | fwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 1.67000000 | 0.05417308 | <.0001 | 1 |
| 0 | 12.5 | 1.66000000 | 0.05417308 | <.0001 | 2 |
| 0 | 18.8 | 1.89000000 | 0.05417308 | <.0001 | 3 |
| 0 | 25 | 1.67333333 | 0.05417308 | <.0001 | 4 |
| 15 | 0 | 1.83000000 | 0.05417308 | <.0001 | 5 |
| 15 | 12.5 | 1.82333333 | 0.05417308 | <.0001 | 6 |
| 15 | 18.8 | 1.72333333 | 0.05417308 | <.0001 | 7 |
| 15 | 25 | 1.76666667 | 0.05417308 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: fwt

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.8978 | 0.0111 | 0.9658 | 0.0531 | 0.0626 | 0.4963 | 0.2251 |
| 2 | 0.8978 | | 0.0084 | 0.8640 | 0.0413 | 0.0489 | 0.4206 | 0.1829 |
| 3 | 0.0111 | 0.0084 | | 0.0121 | 0.4450 | 0.3971 | 0.0449 | 0.1270 |
| 4 | 0.9658 | 0.8640 | 0.0121 | | 0.0577 | 0.0679 | 0.5233 | 0.2408 |
| 5 | 0.0531 | 0.0413 | 0.4450 | 0.0577 | | 0.9317 | 0.1829 | 0.4206 |
| 6 | 0.0626 | 0.0489 | 0.3971 | 0.0679 | 0.9317 | | 0.2103 | 0.4702 |
| 7 | 0.4963 | 0.4206 | 0.0449 | 0.5233 | 0.1829 | 0.2103 | | 0.5795 |
| 8 | 0.2251 | 0.1829 | 0.1270 | 0.2408 | 0.4206 | 0.4702 | 0.5795 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for fwt
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.008804 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.0812 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 1.78583 | 12 | 15 |
| A | | | |
| A | 1.72333 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.008804 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.1148 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 1.80667 | 6 | 18.8 |
| A | | | |
| A | 1.75000 | 6 | 0 |
| A | | | |
| A | 1.74167 | 6 | 12.5 |
| A | | | |
| A | 1.72000 | 6 | 25 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of DDGS | Level of Enz | N | fwt Mean | fwt Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 1.67000000 | 0.06082763 |
| 0 | 12.5 | 3 | 1.66000000 | 0.12489996 |
| 0 | 18.8 | 3 | 1.89000000 | 0.12124356 |
| 0 | 25 | 3 | 1.67333333 | 0.09073772 |
| 15 | 0 | 3 | 1.83000000 | 0.01000000 |
| 15 | 12.5 | 3 | 1.82333333 | 0.11503623 |
| 15 | 18.8 | 3 | 1.72333333 | 0.07637626 |
| 15 | 25 | 3 | 1.76666667 | 0.09504385 |

Body Weight difference

Analysis of 2*4 factorial

The GLM Procedure

Dependent Variable: Bwdif

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 0.07960000 | 0.01137143 | 1.37 | 0.2820 |
| Error | 16 | 0.13253333 | 0.00828333 | | |
| Corrected Total | 23 | 0.21213333 | | | |

| R-Square | Coeff Var | Root MSE | Bwdif Mean |
|---|---|---|---|
| 0.375236 | 124.1084 | 0.091013 | 0.073333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.01500000 | 0.01500000 | 1.81 | 0.1972 |
| Enz | 3 | 0.02143333 | 0.00714444 | 0.86 | 0.4806 |
| DDGS*Enz | 3 | 0.04316667 | 0.01438889 | 1.74 | 0.1997 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.01500000 | 0.01500000 | 1.81 | 0.1972 |
| Enz | 3 | 0.02143333 | 0.00714444 | 0.86 | 0.4806 |
| DDGS*Enz | 3 | 0.04316667 | 0.01438889 | 1.74 | 0.1997 |

| Parameter | | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | | 0.1400000000 B | 0.05254628 | 2.66 | 0.0170 |
| DDGS | 0 | -.1133333333 B | 0.07431166 | -1.53 | 0.1468 |
| DDGS | 15 | 0.0000000000 B | . | . | . |
| Enz | 0 | -.0300000000 B | 0.07431166 | -0.40 | 0.6918 |

| | | | | | |
|---|---|---|---|---|---|
| Enz | 12.5 | -.0900000000 B | 0.07431166 | -1.21 | 0.2434 |
| Enz | 18.8 | -.0466666667 B | 0.07431166 | -0.63 | 0.5389 |
| Enz | 25 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 0 0 | -.0400000000 B | 0.10509255 | -0.38 | 0.7085 |
| DDGS*Enz | 0 12.5 | 0.1366666667 B | 0.10509255 | 1.30 | 0.2119 |
| DDGS*Enz | 0 18.8 | 0.1566666667 B | 0.10509255 | 1.49 | 0.1555 |
| DDGS*Enz | 0 25 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.0000000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial

The GLM Procedure

Least Squares Means

| DDGS | Bwdif LSMEAN | Standard Error | Pr > \|t\| H0:LSMEAN=0 | Pr > \|t\| H0:LSMean1=LSMean2 |
|---|---|---|---|---|
| 0 | 0.04833333 | 0.02627314 | 0.0844 | 0.1972 |
| 15 | 0.09833333 | 0.02627314 | 0.0018 | |

| Enz | Bwdif LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 0.03333333 | 0.03715583 | 0.3830 | 1 |
| 12.5 | 0.06166667 | 0.03715583 | 0.1164 | 2 |
| 18.8 | 0.11500000 | 0.03715583 | 0.0070 | 3 |
| 25 | 0.08333333 | 0.03715583 | 0.0394 | 4 |

Least Squares Means for effect Enz

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Bwdif

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.5972 | 0.1397 | 0.3555 |
| 2 | 0.5972 | | 0.3252 | 0.6856 |
| 3 | 0.1397 | 0.3252 | | 0.5552 |
| 4 | 0.3555 | 0.6856 | 0.5552 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

comparisons should be used.

| DDGS | Enz | Bwdif LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | -0.04333333 | 0.05254628 | 0.4217 | 1 |
| 0 | 12.5 | 0.07333333 | 0.05254628 | 0.1819 | 2 |
| 0 | 18.8 | 0.13666667 | 0.05254628 | 0.0193 | 3 |
| 0 | 25 | 0.02666667 | 0.05254628 | 0.6187 | 4 |
| 15 | 0 | 0.11000000 | 0.05254628 | 0.0526 | 5 |
| 15 | 12.5 | 0.05000000 | 0.05254628 | 0.3555 | 6 |
| 15 | 18.8 | 0.09333333 | 0.05254628 | 0.0947 | 7 |
| 15 | 25 | 0.14000000 | 0.05254628 | 0.0170 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Bwdif

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.1360 | 0.0277 | 0.3602 | 0.0557 | 0.2272 | 0.0845 | 0.0253 |
| 2 | 0.1360 | | 0.4066 | 0.5389 | 0.6284 | 0.7576 | 0.7913 | 0.3830 |
| 3 | 0.0277 | 0.4066 | | 0.1582 | 0.7244 | 0.2606 | 0.5679 | 0.9648 |
| 4 | 0.3602 | 0.5389 | 0.1582 | | 0.2787 | 0.7576 | 0.3830 | 0.1468 |
| 5 | 0.0557 | 0.6284 | 0.7244 | 0.2787 | | 0.4313 | 0.8254 | 0.6918 |
| 6 | 0.2272 | 0.7576 | 0.2606 | 0.7576 | 0.4313 | | 0.5679 | 0.2434 |
| 7 | 0.0845 | 0.7913 | 0.5679 | 0.3830 | 0.8254 | 0.5679 | | 0.5389 |
| 8 | 0.0253 | 0.3830 | 0.9648 | 0.1468 | 0.6918 | 0.2434 | 0.5389 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Bwdif
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.008283 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.0788 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 0.09833 | 12 | 15 |
| A | | | |
| A | 0.04833 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Bwdif
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.008283 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.1114 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 0.11500 | 6 | 18.8 |
| A | | | |
| A | 0.08333 | 6 | 25 |
| A | | | |
| A | 0.06167 | 6 | 12.5 |
| A | | | |
| A | 0.03333 | 6 | 0 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of DDGS | Level of Enz | N | ------------Bwdif------------ Mean | Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | -0.04333333 | 0.12055428 |
| 0 | 12.5 | 3 | 0.07333333 | 0.11930353 |
| 0 | 18.8 | 3 | 0.13666667 | 0.07023769 |
| 0 | 25 | 3 | 0.02666667 | 0.05859465 |
| 15 | 0 | 3 | 0.11000000 | 0.05196152 |
| 15 | 12.5 | 3 | 0.05000000 | 0.08544004 |
| 15 | 18.8 | 3 | 0.09333333 | 0.10016653 |
| 15 | 25 | 3 | 0.14000000 | 0.09539392 |

Number of Eggs

Analysis of 2*4 factorial

The GLM Procedure

Dependent Variable: negg

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 6381.29167 | 911.61310 | 0.38 | 0.9020 |
| Error | 16 | 38592.66667 | 2412.04167 | | |
| Corrected Total | 23 | 44973.95833 | | | |

| R-Square | Coeff Var | Root MSE | negg Mean |
|---|---|---|---|
| 0.141889 | 32.91541 | 49.11254 | 149.2083 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 513.375000 | 513.375000 | 0.21 | 0.6508 |
| Enz | 3 | 3132.458333 | 1044.152778 | 0.43 | 0.7324 |
| DDGS*Enz | 3 | 2735.458333 | 911.819444 | 0.38 | 0.7701 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 513.375000 | 513.375000 | 0.21 | 0.6508 |
| Enz | 3 | 3132.458333 | 1044.152778 | 0.43 | 0.7324 |
| DDGS*Enz | 3 | 2735.458333 | 911.819444 | 0.38 | 0.7701 |

| Parameter | | Estimate | | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| Intercept | | 147.3333333 | B | 28.35513867 | 5.20 | <.0001 |
| DDGS | 0 | -32.0000000 | B | 40.10022167 | -0.80 | 0.4366 |
| DDGS | 15 | 0.0000000 | B | . | . | . |
| Enz | 0 | -9.3333333 | B | 40.10022167 | -0.23 | 0.8189 |

| | | | | | |
|---|---|---|---|---|---|
| Enz | 12.5 | 9.6666667 B | 40.10022167 | 0.24 | 0.8126 |
| Enz | 18.8 | 25.6666667 B | 40.10022167 | 0.64 | 0.5312 |
| Enz | 25 | 0.0000000 B | . | . | . |
| DDGS*Enz | 0 0 | 50.3333333 B | 56.71027734 | 0.89 | 0.3879 |
| DDGS*Enz | 0 12.5 | 36.6666667 B | 56.71027734 | 0.65 | 0.5271 |
| DDGS*Enz | 0 18.8 | 4.0000000 B | 56.71027734 | 0.07 | 0.9446 |
| DDGS*Enz | 0 25 | 0.0000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.0000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.0000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.0000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.0000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial
The GLM Procedure
Least Squares Means

| DDGS | negg LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 144.583333 | 14.177569 | <.0001 | 0.6508 |
| 15 | 153.833333 | 14.177569 | <.0001 | |

| Enz | negg LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 147.166667 | 20.050111 | <.0001 | 1 |
| 12.5 | 159.333333 | 20.050111 | <.0001 | 2 |
| 18.8 | 159.000000 | 20.050111 | <.0001 | 3 |
| 25 | 131.333333 | 20.050111 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: negg

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.6736 | 0.6820 | 0.5843 |
| 2 | 0.6736 | | 0.9908 | 0.3381 |
| 3 | 0.6820 | 0.9908 | | 0.3437 |
| 4 | 0.5843 | 0.3381 | 0.3437 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

comparisons should be used.

| DDGS | Enz | negg LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 156.333333 | 28.355139 | <.0001 | 1 |
| 0 | 12.5 | 161.666667 | 28.355139 | <.0001 | 2 |
| 0 | 18.8 | 145.000000 | 28.355139 | 0.0001 | 3 |
| 0 | 25 | 115.333333 | 28.355139 | 0.0009 | 4 |
| 15 | 0 | 138.000000 | 28.355139 | 0.0002 | 5 |
| 15 | 12.5 | 157.000000 | 28.355139 | <.0001 | 6 |
| 15 | 18.8 | 173.000000 | 28.355139 | <.0001 | 7 |
| 15 | 25 | 147.333333 | 28.355139 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: negg

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.8959 | 0.7811 | 0.3218 | 0.6537 | 0.9869 | 0.6832 | 0.8253 |
| 2 | 0.8959 | | 0.6832 | 0.2649 | 0.5633 | 0.9088 | 0.7811 | 0.7254 |
| 3 | 0.7811 | 0.6832 | | 0.4701 | 0.8636 | 0.7686 | 0.4950 | 0.9543 |
| 4 | 0.3218 | 0.2649 | 0.4701 | | 0.5797 | 0.3142 | 0.1697 | 0.4366 |
| 5 | 0.6537 | 0.5633 | 0.8636 | 0.5797 | | 0.6420 | 0.3957 | 0.8189 |
| 6 | 0.9869 | 0.9088 | 0.7686 | 0.3142 | 0.6420 | | 0.6952 | 0.8126 |
| 7 | 0.6832 | 0.7811 | 0.4950 | 0.1697 | 0.3957 | 0.6952 | | 0.5312 |
| 8 | 0.8253 | 0.7254 | 0.9543 | 0.4366 | 0.8189 | 0.8126 | 0.5312 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for negg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 2412.042 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 42.504 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 153.83 | 12 | 15 |
| A | | | |
| A | 144.58 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for negg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 2412.042 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 60.11 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 159.33 | 6 | 12.5 |
| A | | | |
| A | 159.00 | 6 | 18.8 |
| A | | | |
| A | 147.17 | 6 | 0 |
| A | | | |
| A | 131.33 | 6 | 25 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of DDGS | Level of Enz | N | --------negg-------- Mean | Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 156.333333 | 34.6458271 |
| 0 | 12.5 | 3 | 161.666667 | 92.0072461 |
| 0 | 18.8 | 3 | 145.000000 | 62.7455178 |
| 0 | 25 | 3 | 115.333333 | 23.4591844 |

| | | | | |
|---|---|---|---|---|
| 15 | 0 | 3 | 138.000000 | 14.4222051 |
| 15 | 12.5 | 3 | 157.000000 | 39.2300905 |
| 15 | 18.8 | 3 | 173.000000 | 54.1479455 |
| 15 | 25 | 3 | 147.333333 | 21.5483951 |

## Average Egg Weight

Analysis of 2*4 factorial
The GLM Procedure

Dependent Variable: aveggwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 33.2929167 | 4.7561310 | 0.55 | 0.7813 |
| Error | 16 | 137.2266667 | 8.5766667 | | |
| Corrected Total | 23 | 170.5195833 | | | |

| R-Square | Coeff Var | Root MSE | aveggwt Mean |
|---|---|---|---|
| 0.195244 | 3.812240 | 2.928595 | 76.82083 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.35041667 | 0.35041667 | 0.04 | 0.8424 |
| Enz | 3 | 23.83791667 | 7.94597222 | 0.93 | 0.4506 |
| DDGS*Enz | 3 | 9.10458333 | 3.03486111 | 0.35 | 0.7870 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.35041667 | 0.35041667 | 0.04 | 0.8424 |
| Enz | 3 | 23.83791667 | 7.94597222 | 0.93 | 0.4506 |
| DDGS*Enz | 3 | 9.10458333 | 3.03486111 | 0.35 | 0.7870 |

| Parameter | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Intercept | | 76.90000000 B | 1.69082491 | 45.48 | <.0001 |
| DDGS | 0 | -0.00000000 B | 2.39118752 | -0.00 | 1.0000 |
| DDGS | 15 | 0.00000000 B | . | . | . |
| Enz | 0 | 1.00000000 B | 2.39118752 | 0.42 | 0.6814 |
| Enz | 12.5 | -2.33333333 B | 2.39118752 | -0.98 | 0.3437 |
| Enz | 18.8 | 1.50000000 B | 2.39118752 | 0.63 | 0.5393 |
| Enz | 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 0 0 | -0.06666667 B | 3.38164983 | -0.02 | 0.9845 |
| DDGS*Enz | 0 12.5 | 1.26666667 B | 3.38164983 | 0.37 | 0.7129 |
| DDGS*Enz | 0 18.8 | -2.16666667 B | 3.38164983 | -0.64 | 0.5308 |
| DDGS*Enz | 0 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.00000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial

The GLM Procedure

Least Squares Means

| DDGS | aveggwt LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1=LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 76.7000000 | 0.8454125 | <.0001 | 0.8424 |
| 15 | 76.9416667 | 0.8454125 | <.0001 | |

| Enz | aveggwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 77.8666667 | 1.1955938 | <.0001 | 1 |
| 12.5 | 75.2000000 | 1.1955938 | <.0001 | 2 |
| 18.8 | 77.3166667 | 1.1955938 | <.0001 | 3 |
| 25 | 76.9000000 | 1.1955938 | <.0001 | 4 |

Least Squares Means for effect Enz

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: aveggwt

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.1343 | 0.7492 | 0.5755 |
| 2 | 0.1343 | | 0.2286 | 0.3297 |
| 3 | 0.7492 | 0.2286 | | 0.8085 |
| 4 | 0.5755 | 0.3297 | 0.8085 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | aveggwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 77.8333333 | 1.6908249 | <.0001 | 1 |
| 0 | 12.5 | 75.8333333 | 1.6908249 | <.0001 | 2 |
| 0 | 18.8 | 76.2333333 | 1.6908249 | <.0001 | 3 |
| 0 | 25 | 76.9000000 | 1.6908249 | <.0001 | 4 |
| 15 | 0 | 77.9000000 | 1.6908249 | <.0001 | 5 |
| 15 | 12.5 | 74.5666667 | 1.6908249 | <.0001 | 6 |
| 15 | 18.8 | 78.4000000 | 1.6908249 | <.0001 | 7 |
| 15 | 25 | 76.9000000 | 1.6908249 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: aveggwt

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.4152 | 0.5130 | 0.7014 | 0.9781 | 0.1908 | 0.8157 | 0.7014 |
| 2 | 0.4152 | | 0.8692 | 0.6615 | 0.4002 | 0.6036 | 0.2990 | 0.6615 |
| 3 | 0.5130 | 0.8692 | | 0.7840 | 0.4958 | 0.4958 | 0.3783 | 0.7840 |
| 4 | 0.7014 | 0.6615 | 0.7840 | | 0.6814 | 0.3437 | 0.5393 | 1.0000 |
| 5 | 0.9781 | 0.4002 | 0.4958 | 0.6814 | | 0.1824 | 0.8370 | 0.6814 |
| 6 | 0.1908 | 0.6036 | 0.4958 | 0.3437 | 0.1824 | | 0.1285 | 0.3437 |
| 7 | 0.8157 | 0.2990 | 0.3783 | 0.5393 | 0.8370 | 0.1285 | | 0.5393 |
| 8 | 0.7014 | 0.6615 | 0.7840 | 1.0000 | 0.6814 | 0.3437 | 0.5393 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for aveggwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |

```
            Error Mean Square              8.576667
            Critical Value of t             2.11991
            Least Significant Difference    2.5345
  Means with the same letter are not significantly different.
       t Grouping           Mean       N     DDGS

                A         76.942      12     15
                A
                A         76.700      12     0



                 Analysis of 2*4 factorial
                      The GLM Procedure
                  t Tests (LSD) for aveggwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise
                         error rate.
            Alpha                                  0.05
            Error Degrees of Freedom                 16
            Error Mean Square              8.576667
            Critical Value of t             2.11991
            Least Significant Difference    3.5844
  Means with the same letter are not significantly different.
       t Grouping           Mean       N     Enz

                A         77.867       6     0
                A
                A         77.317       6     18.8
                A
                A         76.900       6     25
                A
                A         75.200       6     12.5

                                Analysis of 2*4 factorial
                      The GLM Procedure
  Level of      Level of              -----------aveggwt-----------
  DDGS          Enz            N               Mean          Std Dev

  0             0              3         77.8333333       3.54729944
  0             12.5           3         75.8333333       0.76376262
  0             18.8           3         76.2333333       3.28836332
  0             25             3         76.9000000       4.15090352
  15            0              3         77.9000000       2.47588368
```

| | | | | |
|---|---|---|---|---|
| 15 | 12.5 | 3 | 74.5666667 | 1.67729942 |
| 15 | 18.8 | 3 | 78.4000000 | 2.51594913 |
| 15 | 25 | 3 | 76.9000000 | 3.48281495 |

## Eggshell weight

Analysis of 2*4 factorial
The GLM Procedure

Dependent Variable: Eshwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 1.87833333 | 0.26833333 | 1.03 | 0.4501 |
| Error | 16 | 4.18000000 | 0.26125000 | | |
| Corrected Total | 23 | 6.05833333 | | | |

| R-Square | Coeff Var | Root MSE | Eshwt Mean |
|---|---|---|---|
| 0.310041 | 4.795555 | 0.511126 | 10.65833 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.16666667 | 0.16666667 | 0.64 | 0.4361 |
| Enz | 3 | 1.18833333 | 0.39611111 | 1.52 | 0.2485 |
| DDGS*Enz | 3 | 0.52333333 | 0.17444444 | 0.67 | 0.5841 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.16666667 | 0.16666667 | 0.64 | 0.4361 |
| Enz | 3 | 1.18833333 | 0.39611111 | 1.52 | 0.2485 |
| DDGS*Enz | 3 | 0.52333333 | 0.17444444 | 0.67 | 0.5841 |

| Parameter | | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | | 10.50000000 B | 0.29509885 | 35.58 | <.0001 |
| DDGS | 0 | -0.03333333 B | 0.41733280 | -0.08 | 0.9373 |
| DDGS | 15 | 0.00000000 B | . | . | . |
| Enz | 0 | 0.56666667 B | 0.41733280 | 1.36 | 0.1934 |
| Enz | 12.5 | 0.06666667 B | 0.41733280 | 0.16 | 0.8751 |
| Enz | 18.8 | 0.33333333 B | 0.41733280 | 0.80 | 0.4361 |
| Enz | 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 0 0 | -0.03333333 B | 0.59019771 | -0.06 | 0.9557 |
| DDGS*Enz | 0 12.5 | 0.13333333 B | 0.59019771 | 0.23 | 0.8241 |
| DDGS*Enz | 0 18.8 | -0.63333333 B | 0.59019771 | -1.07 | 0.2992 |
| DDGS*Enz | 0 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.00000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial
The GLM Procedure
Least Squares Means

| DDGS | Eshwt LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 10.5750000 | 0.1475494 | <.0001 | 0.4361 |
| 15 | 10.7416667 | 0.1475494 | <.0001 | |

| Enz | Eshwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 11.0333333 | 0.2086664 | <.0001 | 1 |
| 12.5 | 10.6166667 | 0.2086664 | <.0001 | 2 |
| 18.8 | 10.5000000 | 0.2086664 | <.0001 | 3 |
| 25 | 10.4833333 | 0.2086664 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Eshwt

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.1771 | 0.0896 | 0.0808 |
| 2 | 0.1771 | | 0.6978 | 0.6575 |
| 3 | 0.0896 | 0.6978 | | 0.9557 |
| 4 | 0.0808 | 0.6575 | 0.9557 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | Eshwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 11.0000000 | 0.2950989 | <.0001 | 1 |
| 0 | 12.5 | 10.6666667 | 0.2950989 | <.0001 | 2 |
| 0 | 18.8 | 10.1666667 | 0.2950989 | <.0001 | 3 |
| 0 | 25 | 10.4666667 | 0.2950989 | <.0001 | 4 |
| 15 | 0 | 11.0666667 | 0.2950989 | <.0001 | 5 |
| 15 | 12.5 | 10.5666667 | 0.2950989 | <.0001 | 6 |
| 15 | 18.8 | 10.8333333 | 0.2950989 | <.0001 | 7 |
| 15 | 25 | 10.5000000 | 0.2950989 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Eshwt

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.4361 | 0.0631 | 0.2195 | 0.8751 | 0.3145 | 0.6949 | 0.2483 |
| 2 | 0.4361 | | 0.2483 | 0.6383 | 0.3521 | 0.8137 | 0.6949 | 0.6949 |
| 3 | 0.0631 | 0.2483 | | 0.4826 | 0.0466 | 0.3521 | 0.1297 | 0.4361 |
| 4 | 0.2195 | 0.6383 | 0.4826 | | 0.1698 | 0.8137 | 0.3926 | 0.9373 |
| 5 | 0.8751 | 0.3521 | 0.0466 | 0.1698 | | 0.2483 | 0.5838 | 0.1934 |
| 6 | 0.3145 | 0.8137 | 0.3521 | 0.8137 | 0.2483 | | 0.5319 | 0.8751 |
| 7 | 0.6949 | 0.6949 | 0.1297 | 0.3926 | 0.5838 | 0.5319 | | 0.4361 |
| 8 | 0.2483 | 0.6949 | 0.4361 | 0.9373 | 0.1934 | 0.8751 | 0.4361 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Eshwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.26125 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.4424 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 10.7417 | 12 | 15 |
| A | | | |
| A | 10.5750 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Eshwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.26125 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.6256 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 11.0333 | 6 | 0 |
| A | | | |
| A | 10.6167 | 6 | 12.5 |
| A | | | |

| | | | |
|---|---|---|---|
| A | 10.5000 | 6 | 18.8 |
| A | | | |
| A | 10.4833 | 6 | 25 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of DDGS | Level of Enz | N | Eshwt Mean | Eshwt Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 11.0000000 | 0.00000000 |
| 0 | 12.5 | 3 | 10.6666667 | 0.57735027 |
| 0 | 18.8 | 3 | 10.1666667 | 0.57735027 |
| 0 | 25 | 3 | 10.4666667 | 0.40414519 |
| 15 | 0 | 3 | 11.0666667 | 0.40414519 |
| 15 | 12.5 | 3 | 10.5666667 | 0.51316014 |
| 15 | 18.8 | 3 | 10.8333333 | 0.28867513 |
| 15 | 25 | 3 | 10.5000000 | 0.86602540 |

## Eggshell thickness

Analysis of 2*4 factorial

The GLM Procedure

Dependent Variable: eshT

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 0.00069896 | 0.00009985 | 0.24 | 0.9684 |
| Error | 16 | 0.00665433 | 0.00041590 | | |
| Corrected Total | 23 | 0.00735329 | | | |

| R-Square | Coeff Var | Root MSE | eshT Mean |
|---|---|---|---|
| 0.095053 | 5.508507 | 0.020394 | 0.370219 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| DDGS | 1 | 0.00000250 | 0.00000250 | 0.01 | 0.9391 |
| Enz | 3 | 0.00053292 | 0.00017764 | 0.43 | 0.7363 |
| DDGS*Enz | 3 | 0.00016353 | 0.00005451 | 0.13 | 0.9402 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.00000250 | 0.00000250 | 0.01 | 0.9391 |
| Enz | 3 | 0.00053292 | 0.00017764 | 0.43 | 0.7363 |
| DDGS*Enz | 3 | 0.00016353 | 0.00005451 | 0.13 | 0.9402 |

| Parameter | | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | | 0.3695833333 B | 0.01177421 | 31.39 | <.0001 |
| DDGS | 0 | -.0070833333 B | 0.01665124 | -0.43 | 0.6762 |
| DDGS | 15 | 0.0000000000 B | . | . | . |
| Enz | 0 | 0.0066666667 B | 0.01665124 | 0.40 | 0.6942 |
| Enz | 12.5 | 0.0021666667 B | 0.01665124 | 0.13 | 0.8981 |
| Enz | 18.8 | -.0050000000 B | 0.01665124 | -0.30 | 0.7678 |
| Enz | 25 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 0 0 | 0.0107500000 B | 0.02354841 | 0.46 | 0.6542 |
| DDGS*Enz | 0 12.5 | 0.0026666667 B | 0.02354841 | 0.11 | 0.9112 |
| DDGS*Enz | 0 18.8 | 0.0123333333 B | 0.02354841 | 0.52 | 0.6076 |
| DDGS*Enz | 0 25 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.0000000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.0000000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial

The GLM Procedure

Least Squares Means

| DDGS | eshT LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 0 | 0.36989583 | 0.00588710 | <.0001 | 0.9391 |
| 15 | 0.37054167 | 0.00588710 | <.0001 | |

| Enz | eshT LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 0.37808333 | 0.00832562 | <.0001 | 1 |
| 12.5 | 0.36954167 | 0.00832562 | <.0001 | 2 |
| 18.8 | 0.36720833 | 0.00832562 | <.0001 | 3 |
| 25 | 0.36604167 | 0.00832562 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: eshT

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.4786 | 0.3694 | 0.3217 |
| 2 | 0.4786 | | 0.8454 | 0.7701 |
| 3 | 0.3694 | 0.8454 | | 0.9223 |
| 4 | 0.3217 | 0.7701 | 0.9223 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | eshT LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 0.37991667 | 0.01177421 | <.0001 | 1 |
| 0 | 12.5 | 0.36733333 | 0.01177421 | <.0001 | 2 |
| 0 | 18.8 | 0.36983333 | 0.01177421 | <.0001 | 3 |
| 0 | 25 | 0.36250000 | 0.01177421 | <.0001 | 4 |
| 15 | 0 | 0.37625000 | 0.01177421 | <.0001 | 5 |
| 15 | 12.5 | 0.37175000 | 0.01177421 | <.0001 | 6 |
| 15 | 18.8 | 0.36458333 | 0.01177421 | <.0001 | 7 |
| 15 | 25 | 0.36958333 | 0.01177421 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: eshT

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.4608 | 0.5533 | 0.3111 | 0.8285 | 0.6305 | 0.3708 | 0.5436 |
| 2 | 0.4608 | | 0.8825 | 0.7753 | 0.5997 | 0.7942 | 0.8709 | 0.8942 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3 | 0.5533 | 0.8825 | | 0.6655 | 0.7050 | 0.9098 | 0.7566 | 0.9882 |
| 4 | 0.3111 | 0.7753 | 0.6655 | | 0.4211 | 0.5862 | 0.9020 | 0.6762 |
| 5 | 0.8285 | 0.5997 | 0.7050 | 0.4211 | | 0.7904 | 0.4936 | 0.6942 |
| 6 | 0.6305 | 0.7942 | 0.9098 | 0.5862 | 0.7904 | | 0.6726 | 0.8981 |
| 7 | 0.3708 | 0.8709 | 0.7566 | 0.9020 | 0.4936 | 0.6726 | | 0.7678 |
| 8 | 0.5436 | 0.8942 | 0.9882 | 0.6762 | 0.6942 | 0.8981 | 0.7678 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial

The GLM Procedure

t Tests (LSD) for eshT

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.000416 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.0176 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 0.370542 | 12 | 15 |
| A | | | |
| A | 0.369896 | 12 | 0 |

Analysis of 2*4 factorial

The GLM Procedure

t Tests (LSD) for eshT

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.000416 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.025 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 0.37808 | 6 | 0 |
| A | | | |
| A | 0.36954 | 6 | 12.5 |
| A | | | |
| A | 0.36721 | 6 | 18.8 |
| A | | | |
| A | 0.36604 | 6 | 25 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of DDGS | Level of Enz | N | ------------eshT------------ Mean | Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 0.37991667 | 0.00988791 |
| 0 | 12.5 | 3 | 0.36733333 | 0.01854948 |
| 0 | 18.8 | 3 | 0.36983333 | 0.01317510 |
| 0 | 25 | 3 | 0.36250000 | 0.00965660 |
| 15 | 0 | 3 | 0.37625000 | 0.02116453 |
| 15 | 12.5 | 3 | 0.37175000 | 0.03170863 |
| 15 | 18.8 | 3 | 0.36458333 | 0.03014272 |
| 15 | 25 | 3 | 0.36958333 | 0.01601627 |

## Albumen Weight

Analysis of 2*4 factorial

The GLM Procedure

Dependent Variable: Albwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 35.3783333 | 5.0540476 | 0.72 | 0.6570 |
| Error | 16 | 112.2600000 | 7.0162500 | | |
| Corrected Total | 23 | 147.6383333 | | | |

| R-Square | Coeff Var | Root MSE | Albwt Mean |
|---|---|---|---|
| 0.239628 | 5.715851 | 2.648820 | 46.34167 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| DDGS | 1 | 0.32666667 | 0.32666667 | 0.05 | 0.8319 |
| Enz | 3 | 24.08833333 | 8.02944444 | 1.14 | 0.3613 |
| DDGS*Enz | 3 | 10.96333333 | 3.65444444 | 0.52 | 0.6740 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 0.32666667 | 0.32666667 | 0.05 | 0.8319 |
| Enz | 3 | 24.08833333 | 8.02944444 | 1.14 | 0.3613 |
| DDGS*Enz | 3 | 10.96333333 | 3.65444444 | 0.52 | 0.6740 |

| Parameter | | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | | 46.33333333 B | 1.52929722 | 30.30 | <.0001 |
| DDGS | 0 | 1.16666667 B | 2.16275288 | 0.54 | 0.5970 |
| DDGS | 15 | 0.00000000 B | . | . | . |
| Enz | 0 | 0.83333333 B | 2.16275288 | 0.39 | 0.7051 |
| Enz | 12.5 | -2.16666667 B | 2.16275288 | -1.00 | 0.3313 |
| Enz | 18.8 | 1.83333333 B | 2.16275288 | 0.85 | 0.4091 |
| Enz | 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 0 0 | -2.66666667 B | 3.05859445 | -0.87 | 0.3962 |
| DDGS*Enz | 0 12.5 | -0.10000000 B | 3.05859445 | -0.03 | 0.9743 |
| DDGS*Enz | 0 18.8 | -2.83333333 B | 3.05859445 | -0.93 | 0.3680 |
| DDGS*Enz | 0 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.00000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial
The GLM Procedure
Least Squares Means

| DDGS | Albwt LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 46.2250000 | 0.7646486 | <.0001 | 0.8319 |

| | | | |
|---|---|---|---|
| 15 | 46.4583333 | 0.7646486 | <.0001 |

| Enz | Albwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 46.4166667 | 1.0813764 | <.0001 | 1 |
| 12.5 | 44.7000000 | 1.0813764 | <.0001 | 2 |
| 18.8 | 47.3333333 | 1.0813764 | <.0001 | 3 |
| 25 | 46.9166667 | 1.0813764 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Albwt

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.2782 | 0.5573 | 0.7479 |
| 2 | 0.2782 | | 0.1044 | 0.1665 |
| 3 | 0.5573 | 0.1044 | | 0.7888 |
| 4 | 0.7479 | 0.1665 | 0.7888 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | Albwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 45.6666667 | 1.5292972 | <.0001 | 1 |
| 0 | 12.5 | 45.2333333 | 1.5292972 | <.0001 | 2 |
| 0 | 18.8 | 46.5000000 | 1.5292972 | <.0001 | 3 |
| 0 | 25 | 47.5000000 | 1.5292972 | <.0001 | 4 |
| 15 | 0 | 47.1666667 | 1.5292972 | <.0001 | 5 |
| 15 | 12.5 | 44.1666667 | 1.5292972 | <.0001 | 6 |
| 15 | 18.8 | 48.1666667 | 1.5292972 | <.0001 | 7 |
| 15 | 25 | 46.3333333 | 1.5292972 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Albwt

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.8437 | 0.7051 | 0.4091 | 0.4979 | 0.4979 | 0.2647 | 0.7619 |
| 2 | 0.8437 | | 0.5663 | 0.3102 | 0.3846 | 0.6286 | 0.1938 | 0.6180 |
| 3 | 0.7051 | 0.5663 | | 0.6500 | 0.7619 | 0.2966 | 0.4522 | 0.9395 |
| 4 | 0.4091 | 0.3102 | 0.6500 | | 0.8794 | 0.1428 | 0.7619 | 0.5970 |
| 5 | 0.4979 | 0.3846 | 0.7619 | 0.8794 | | 0.1844 | 0.6500 | 0.7051 |
| 6 | 0.4979 | 0.6286 | 0.2966 | 0.1428 | 0.1844 | | 0.0829 | 0.3313 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 7 | 0.2647 | 0.1938 | 0.4522 | 0.7619 | 0.6500 | 0.0829 | | 0.4091 |
| 8 | 0.7619 | 0.6180 | 0.9395 | 0.5970 | 0.7051 | 0.3313 | 0.4091 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Albwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 7.01625 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 2.2924 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 46.458 | 12 | 15 |
| A | | | |
| A | 46.225 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Albwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 7.01625 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 3.242 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|

| | | | |
|---|---|---|---|
| A | 47.333 | 6 | 18.8 |
| A | | | |
| A | 46.917 | 6 | 25 |
| A | | | |
| A | 46.417 | 6 | 0 |
| A | | | |
| A | 44.700 | 6 | 12.5 |

Analysis of 2*4 factorial

The GLM Procedure

| Level of DDGS | Level of Enz | N | ------------Albwt------------ Mean | Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 45.6666667 | 2.30940108 |
| 0 | 12.5 | 3 | 45.2333333 | 0.68068593 |
| 0 | 18.8 | 3 | 46.5000000 | 2.17944947 |
| 0 | 25 | 3 | 47.5000000 | 4.76969601 |
| 15 | 0 | 3 | 47.1666667 | 1.75594229 |
| 15 | 12.5 | 3 | 44.1666667 | 2.46644143 |
| 15 | 18.8 | 3 | 48.1666667 | 2.30940108 |
| 15 | 25 | 3 | 46.3333333 | 2.88675135 |

# Yolk Weight

Analysis of 2*4 factorial

The GLM Procedure

Dependent Variable: yolkwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 2.60625000 | 0.37232143 | 1.05 | 0.4374 |

| | | | |
|---|---|---|---|
| Error | 16 | 5.68000000 | 0.35500000 |
| Corrected Total | 23 | 8.28625000 | |

| R-Square | Coeff Var | Root MSE | yolkwt Mean |
|---|---|---|---|
| 0.314527 | 3.201175 | 0.595819 | 18.61250 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 1.26041667 | 1.26041667 | 3.55 | 0.0778 |
| Enz | 3 | 0.54125000 | 0.18041667 | 0.51 | 0.6822 |
| DDGS*Enz | 3 | 0.80458333 | 0.26819444 | 0.76 | 0.5352 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 1.26041667 | 1.26041667 | 3.55 | 0.0778 |
| Enz | 3 | 0.54125000 | 0.18041667 | 0.51 | 0.6822 |
| DDGS*Enz | 3 | 0.80458333 | 0.26819444 | 0.76 | 0.5352 |

| Parameter | | Estimate | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|
| Intercept | | 18.73333333 B | 0.34399612 | 54.46 | <.0001 |
| DDGS | 0 | 0.03333333 B | 0.48648398 | 0.07 | 0.9462 |
| DDGS | 15 | 0.00000000 B | . | . | . |
| Enz | 0 | -0.33333333 B | 0.48648398 | -0.69 | 0.5030 |
| Enz | 12.5 | -0.66666667 B | 0.48648398 | -1.37 | 0.1895 |
| Enz | 18.8 | -0.40000000 B | 0.48648398 | -0.82 | 0.4230 |
| Enz | 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 0 0 | 0.70000000 B | 0.68799225 | 1.02 | 0.3241 |
| DDGS*Enz | 0 12.5 | 0.86666667 B | 0.68799225 | 1.26 | 0.2258 |
| DDGS*Enz | 0 18.8 | 0.13333333 B | 0.68799225 | 0.19 | 0.8488 |
| DDGS*Enz | 0 25 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.00000000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.00000000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial
The GLM Procedure
Least Squares Means

| DDGS | yolkwt LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1= LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 18.8416667 | 0.1719981 | <.0001 | 0.0778 |
| 15 | 18.3833333 | 0.1719981 | <.0001 | |

| Enz | yolkwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 18.7666667 | 0.2432420 | <.0001 | 1 |
| 12.5 | 18.5166667 | 0.2432420 | <.0001 | 2 |
| 18.8 | 18.4166667 | 0.2432420 | <.0001 | 3 |
| 25 | 18.7500000 | 0.2432420 | <.0001 | 4 |

Least Squares Means for effect Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: yolkwt

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.4779 | 0.3241 | 0.9620 |
| 2 | 0.4779 | | 0.7750 | 0.5073 |
| 3 | 0.3241 | 0.7750 | | 0.3470 |
| 4 | 0.9620 | 0.5073 | 0.3470 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | yolkwt LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 19.1333333 | 0.3439961 | <.0001 | 1 |
| 0 | 12.5 | 18.9666667 | 0.3439961 | <.0001 | 2 |
| 0 | 18.8 | 18.5000000 | 0.3439961 | <.0001 | 3 |
| 0 | 25 | 18.7666667 | 0.3439961 | <.0001 | 4 |
| 15 | 0 | 18.4000000 | 0.3439961 | <.0001 | 5 |
| 15 | 12.5 | 18.0666667 | 0.3439961 | <.0001 | 6 |
| 15 | 18.8 | 18.3333333 | 0.3439961 | <.0001 | 7 |
| 15 | 25 | 18.7333333 | 0.3439961 | <.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: yolkwt

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.7364 | 0.2114 | 0.4620 | 0.1512 | 0.0435 | 0.1196 | 0.4230 |
| 2 | 0.7364 | | 0.3517 | 0.6864 | 0.2612 | 0.0829 | 0.2114 | 0.6380 |
| 3 | 0.2114 | 0.3517 | | 0.5912 | 0.8397 | 0.3863 | 0.7364 | 0.6380 |
| 4 | 0.4620 | 0.6864 | 0.5912 | | 0.4620 | 0.1695 | 0.3863 | 0.9462 |
| 5 | 0.1512 | 0.2612 | 0.8397 | 0.4620 | | 0.5030 | 0.8927 | 0.5030 |
| 6 | 0.0435 | 0.0829 | 0.3863 | 0.1695 | 0.5030 | | 0.5912 | 0.1895 |
| 7 | 0.1196 | 0.2114 | 0.7364 | 0.3863 | 0.8927 | 0.5912 | | 0.4230 |
| 8 | 0.4230 | 0.6380 | 0.6380 | 0.9462 | 0.5030 | 0.1895 | 0.4230 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for yolkwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.355 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.5156 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 18.8417 | 12 | 0 |
| A | | | |
| A | 18.3833 | 12 | 15 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for yolkwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 0.355 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 0.7292 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 18.7667 | 6 | 0 |
| A | | | |
| A | 18.7500 | 6 | 25 |
| A | | | |
| A | 18.5167 | 6 | 12.5 |
| A | | | |
| A | 18.4167 | 6 | 18.8 |

Analysis of 2*4 factorial

The GLM Procedure

| Level of DDGS | Level of Enz | N | ------------yolkwt----------- Mean | Std Dev |
|---|---|---|---|---|
| 0 | 0 | 3 | 19.1333333 | 0.51316014 |
| 0 | 12.5 | 3 | 18.9666667 | 0.57735027 |
| 0 | 18.8 | 3 | 18.5000000 | 0.91651514 |
| 0 | 25 | 3 | 18.7666667 | 0.68068593 |
| 15 | 0 | 3 | 18.4000000 | 0.36055513 |
| 15 | 12.5 | 3 | 18.0666667 | 0.81445278 |
| 15 | 18.8 | 3 | 18.3333333 | 0.28867513 |
| 15 | 25 | 3 | 18.7333333 | 0.25166115 |

## Egg Mass

Analysis of 2*4 factorial
The GLM Procedure

Dependent Variable: Eggmass

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 7 | 38698486.1 | 5528355.2 | 0.38 | 0.9022 |
| Error | 16 | 234309085.9 | 14644317.9 | | |
| Corrected Total | 23 | 273007572.0 | | | |

| R-Square | Coeff Var | Root MSE | Eggmass Mean |
|---|---|---|---|
| 0.141749 | 33.36102 | 3826.789 | 11470.84 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 3326277.13 | 3326277.13 | 0.23 | 0.6401 |
| Enz | 3 | 17384369.06 | 5794789.69 | 0.40 | 0.7579 |
| DDGS*Enz | 3 | 17987839.90 | 5995946.63 | 0.41 | 0.7484 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| DDGS | 1 | 3326277.13 | 3326277.13 | 0.23 | 0.6401 |
| Enz | 3 | 17384369.06 | 5794789.69 | 0.40 | 0.7579 |
| DDGS*Enz | 3 | 17987839.90 | 5995946.63 | 0.41 | 0.7484 |

| Parameter | | Estimate | | Standard Error | t Value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| Intercept | | 11326.60000 | B | 2209.397947 | 5.13 | 0.0001 |
| DDGS | 0 | -2415.60000 | B | 3124.560542 | -0.77 | 0.4507 |
| DDGS | 15 | 0.00000 | B | . | . | . |
| Enz | 0 | -586.66667 | B | 3124.560542 | -0.19 | 0.8534 |
| Enz | 12.5 | 365.36667 | B | 3124.560542 | 0.12 | 0.9084 |
| Enz | 18.8 | 2287.40000 | B | 3124.560542 | 0.73 | 0.4747 |

| | | | | | |
|---|---|---|---|---|---|
| Enz | 25 | 0.00000 B | . | . | . |
| DDGS*Enz | 0 0 | 3763.83333 B | 4418.795894 | 0.85 | 0.4069 |
| DDGS*Enz | 0 12.5 | 2998.13333 B | 4418.795894 | 0.68 | 0.5072 |
| DDGS*Enz | 0 18.8 | -77.83333 B | 4418.795894 | -0.02 | 0.9862 |
| DDGS*Enz | 0 25 | 0.00000 B | . | . | . |
| DDGS*Enz | 15 0 | 0.00000 B | . | . | . |
| DDGS*Enz | 15 12.5 | 0.00000 B | . | . | . |
| DDGS*Enz | 15 18.8 | 0.00000 B | . | . | . |
| DDGS*Enz | 15 25 | 0.00000 B | . | . | . |

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Analysis of 2*4 factorial

The GLM Procedure

Least Squares Means

| DDGS | Eggmass LSMEAN | Standard Error | H0:LSMEAN=0 Pr > \|t\| | H0:LSMean1=LSMean2 Pr > \|t\| |
|---|---|---|---|---|
| 0 | 11098.5583 | 1104.6990 | <.0001 | 0.6401 |
| 15 | 11843.1250 | 1104.6990 | <.0001 | |

| Enz | Eggmass LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|
| 0 | 11414.0500 | 1562.2803 | <.0001 | 1 |
| 12.5 | 11983.2333 | 1562.2803 | <.0001 | 2 |
| 18.8 | 12367.2833 | 1562.2803 | <.0001 | 3 |
| 25 | 10118.8000 | 1562.2803 | <.0001 | 4 |

Least Squares Means for effect Enz

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Eggmass

| i/j | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 0.8000 | 0.6719 | 0.5659 |
| 2 | 0.8000 | | 0.8642 | 0.4112 |
| 3 | 0.6719 | 0.8642 | | 0.3240 |
| 4 | 0.5659 | 0.4112 | 0.3240 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

| DDGS | Enz | Eggmass LSMEAN | Standard Error | Pr > \|t\| | LSMEAN Number |
|---|---|---|---|---|---|
| 0 | 0 | 12088.1667 | 2209.3979 | <.0001 | 1 |
| 0 | 12.5 | 12274.5000 | 2209.3979 | <.0001 | 2 |
| 0 | 18.8 | 11120.5667 | 2209.3979 | 0.0001 | 3 |
| 0 | 25 | 8911.0000 | 2209.3979 | 0.0010 | 4 |
| 15 | 0 | 10739.9333 | 2209.3979 | 0.0002 | 5 |
| 15 | 12.5 | 11691.9667 | 2209.3979 | <.0001 | 6 |
| 15 | 18.8 | 13614.0000 | 2209.3979 | <.0001 | 7 |
| 15 | 25 | 11326.6000 | 2209.3979 | 0.0001 | 8 |

Least Squares Means for effect DDGS*Enz
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Eggmass

| i/j | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | 0.9532 | 0.7608 | 0.3244 | 0.6719 | 0.9007 | 0.6319 | 0.8105 |
| 2 | 0.9532 | | 0.7167 | 0.2977 | 0.6300 | 0.8544 | 0.6739 | 0.7655 |
| 3 | 0.7608 | 0.7167 | | 0.4896 | 0.9046 | 0.8572 | 0.4365 | 0.9482 |
| 4 | 0.3244 | 0.2977 | 0.4896 | | 0.5665 | 0.3866 | 0.1518 | 0.4507 |
| 5 | 0.6719 | 0.6300 | 0.9046 | 0.5665 | | 0.7645 | 0.3713 | 0.8534 |
| 6 | 0.9007 | 0.8544 | 0.8572 | 0.3866 | 0.7645 | | 0.5471 | 0.9084 |
| 7 | 0.6319 | 0.6739 | 0.4365 | 0.1518 | 0.3713 | 0.5471 | | 0.4747 |
| 8 | 0.8105 | 0.7655 | 0.9482 | 0.4507 | 0.8534 | 0.9084 | 0.4747 | |

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Eggmass

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 14644318 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 3311.9 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | DDGS |
|---|---|---|---|
| A | 11843 | 12 | 15 |
| A | | | |
| A | 11099 | 12 | 0 |

Analysis of 2*4 factorial
The GLM Procedure
t Tests (LSD) for Eggmass

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 16 |
| Error Mean Square | 14644318 |
| Critical Value of t | 2.11991 |
| Least Significant Difference | 4683.7 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 12367 | 6 | 18.8 |
| A | | | |
| A | 11983 | 6 | 12.5 |
| A | | | |
| A | 11414 | 6 | 0 |
| A | | | |
| A | 10119 | 6 | 25 |

Analysis of 2*4 factorial
The GLM Procedure

| Level of | Level of | | -----------Eggmass----------- | |
|---|---|---|---|---|
| DDGS | Enz | N | Mean | Std Dev |
| 0 | 0 | 3 | 12088.1667 | 2100.62516 |
| 0 | 12.5 | 3 | 12274.5000 | 7013.68771 |
| 0 | 18.8 | 3 | 11120.5667 | 5134.13201 |
| 0 | 25 | 3 | 8911.0000 | 2200.30816 |
| 15 | 0 | 3 | 10739.9333 | 1003.04198 |
| 15 | 12.5 | 3 | 11691.9667 | 2819.37692 |
| 15 | 18.8 | 3 | 13614.0000 | 4513.57057 |
| 15 | 25 | 3 | 11326.6000 | 1738.42903 |

# Annex (3)
# Statistical Analysis (Experiment Two)

Analysis of CRD

| Obs | Enz | inwt | fwt | negg | aveggwt | Eshwt | eshT | Albwt | yolkwt | Bwdif | Eggmass |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 1.632 | 1.858 | 76 | 79 | 10 | 0.327 | 49 | 19 | 0.226 | 6004 |
| 2 | 0 | 1.736 | 1.804 | 77 | 74 | 11 | 0.348 | 44 | 20 | 0.068 | 5698 |
| 3 | 0 | 1.796 | 1.896 | 71 | 75 | 10 | 0.312 | 47 | 20 | 0.100 | 5325 |
| 4 | 100 | 1.830 | 1.748 | 56 | 76 | 10 | 0.348 | 47 | 20 | -0.082 | 4256 |
| 5 | 100 | 1.826 | 1.754 | 76 | 73 | 10 | 0.330 | 45 | 19 | -0.072 | 5548 |
| 6 | 100 | 1.850 | 1.896 | 62 | 77 | 10 | 0.340 | 49 | 19 | 0.046 | 4774 |
| 7 | 200 | 1.770 | 1.804 | 51 | 78 | 10 | 0.337 | 49 | 20 | 0.034 | 3978 |
| 8 | 200 | 1.662 | 1.918 | 54 | 74 | 10 | 0.362 | 45 | 20 | 0.256 | 3996 |
| 9 | 200 | 1.708 | 1.842 | 52 | 77 | 10 | 0.312 | 52 | 18 | 0.134 | 4004 |
| 10 | 500 | 2.028 | 1.896 | 52 | 71 | 10 | 0.352 | 44 | 20 | -0.132 | 3692 |
| 11 | 500 | 1.783 | 1.688 | 67 | 77 | 10 | 0.353 | 48 | 20 | -0.095 | 5159 |
| 12 | 500 | 1.754 | 1.786 | 84 | 74 | 10 | 0.319 | 45 | 20 | 0.032 | 6216 |
| 13 | 1000 | 1.803 | 1.676 | 76 | 73 | 10 | 0.319 | 46 | 20 | -0.127 | 5548 |
| 14 | 1000 | 1.617 | 1.712 | 81 | 77 | 10 | 0.336 | 47 | 19 | 0.095 | 6237 |
| 15 | 1000 | 1.599 | 1.796 | 103 | 75 | 10 | 0.324 | 47 | 19 | 0.197 | 7725 |

Analysis of CRD

The GLM Procedure

Class Level Information

| Class | Levels | Values |
|---|---|---|
| Enz | 5 | 0 100 1000 200 500 |

Number of observations 15

Analysis of CRD

The GLM Procedure

Dependent Variable: inwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 0.07782360 | 0.01945590 | 2.14 | 0.1498 |
| Error | 10 | 0.09080200 | 0.00908020 | | |
| Corrected Total | 14 | 0.16862560 | | | |

| R-Square | CoeffVar | Root MSE | inwt Mean |
|---|---|---|---|
| 0.461517 | 5.415440 | 0.095290 | 1.759600 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.07782360 | 0.01945590 | 2.14 | 0.1498 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.07782360 | 0.01945590 | 2.14 | 0.1498 |

Initial Weight

Analysis of CRD

The GLM Procedure

t Tests (LSD) for inwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 0.00908 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 0.1734 |

Means with the same letter are not significantly different.

| t Grouping | | Mean | N | Enz |
|---|---|---|---|---|
| | A | 1.85500 | 3 | 500 |
| | A | | | |
| B | A | 1.83533 | 3 | 100 |
| B | A | | | |
| B | A | 1.72133 | 3 | 0 |
| B | A | | | |
| B | A | 1.71333 | 3 | 200 |
| B | | | | |
| B | | 1.67300 | 3 | 1000 |

Final Weight

Analysis of CRD

The GLM Procedure

Dependent Variable: fwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 0.03277493 | 0.00819373 | 1.51 | 0.2717 |
| Error | 10 | 0.05428800 | 0.00542880 | | |
| Corrected Total | 14 | 0.08706293 | | | |

| R-Square | CoeffVar | Root MSE | fwt Mean |
|---|---|---|---|
| 0.376451 | 4.082167 | 0.073680 | 1.804933 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.03277493 | 0.00819373 | 1.51 | 0.2717 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.03277493 | 0.00819373 | 1.51 | 0.2717 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 0.005429 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 0.134 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 1.85467 | 3 | 200 |
| A | | | |
| A | 1.85267 | 3 | 0 |
| A | | | |
| A | 1.79933 | 3 | 100 |
| A | | | |
| A | 1.79000 | 3 | 500 |
| A | | | |
| A | 1.72800 | 3 | 1000 |

Body Weight Difference

Analysis of CRD

The GLM Procedure

Dependent Variable: Bwdif

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 0.10648200 | 0.02662050 | 2.25 | 0.1365 |
| Error | 10 | 0.11849933 | 0.01184993 | | |
| Corrected Total | 14 | 0.22498133 | | | |

| R-Square | CoeffVar | Root MSE | Bwdif Mean |
|---|---|---|---|
| 0.473293 | 240.1266 | 0.108857 | 0.045333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.10648200 | 0.02662050 | 2.25 | 0.1365 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.10648200 | 0.02662050 | 2.25 | 0.1365 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for Bwdif

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 0.01185 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 0.198 |

Means with the same letter are not significantly different.

| t Grouping | | Mean | N | Enz |
|---|---|---|---|---|
| | A | 0.14133 | 3 | 200 |
| | A | | | |
| B | A | 0.13133 | 3 | 0 |
| B | A | | | |
| B | A | 0.05500 | 3 | 1000 |
| B | A | | | |
| B | A | -0.03600 | 3 | 100 |
| B | | | | |
| B | | -0.06500 | 3 | 500 |

Number of Eggs

Analysis of CRD

The GLM Procedure

Dependent Variable: negg

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 1927.066667 | 481.766667 | 4.15 | 0.0310 |
| Error | 10 | 1161.333333 | 116.133333 | | |
| Corrected Total | 14 | 3088.400000 | | | |

| R-Square | CoeffVar | Root MSE | negg Mean |
|---|---|---|---|
| 0.623969 | 15.57300 | 10.77652 | 69.20000 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 1927.066667 | 481.766667 | 4.15 | 0.0310 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 1927.066667 | 481.766667 | 4.15 | 0.0310 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for negg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 116.1333 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 19.605 |

Means with the same letter are not significantly different.

| t Grouping | | | Mean | N | Enz |
|---|---|---|---|---|---|
| | A | | 86.667 | 3 | 1000 |
| | A | | | | |
| B | A | | 74.667 | 3 | 0 |
| B | A | | | | |
| B | A | C | 67.667 | 3 | 500 |
| B | | C | | | |
| B | | C | 64.667 | 3 | 100 |
| | | C | | | |
| | | C | 52.333 | 3 | 200 |

Average Egg Weight

Analysis of CRD

The GLM Procedure

Dependent Variable: aveggwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 10.00000000 | 2.50000000 | 0.44 | 0.7800 |
| Error | 10 | 57.33333333 | 5.73333333 | | |
| Corrected Total | 14 | 67.33333333 | | | |

| R-Square | CoeffVar | Root MSE | aveggwt Mean |
|---|---|---|---|
| 0.148515 | 3.178458 | 2.394438 | 75.33333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 10.00000000 | 2.50000000 | 0.44 | 0.7800 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 10.00000000 | 2.50000000 | 0.44 | 0.7800 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for aveggwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 5.733333 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 4.3561 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 76.333 | 3 | 200 |
| A | | | |
| A | 76.000 | 3 | 0 |
| A | | | |
| A | 75.333 | 3 | 100 |
| A | | | |
| A | 75.000 | 3 | 1000 |
| A | | | |
| A | 74.000 | 3 | 500 |

Eggshell Weight

Analysis of CRD

The GLM Procedure

Dependent Variable: Eshwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 0.26666667 | 0.06666667 | 1.00 | 0.4516 |
| Error | 10 | 0.66666667 | 0.06666667 | | |
| Corrected Total | 14 | 0.93333333 | | | |

| R-Square | CoeffVar | Root MSE | Eshwt Mean |
|---|---|---|---|
| 0.285714 | 2.564890 | 0.258199 | 10.06667 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.26666667 | 0.06666667 | 1.00 | 0.4516 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.26666667 | 0.06666667 | 1.00 | 0.4516 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for Eshwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 0.066667 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 0.4697 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 10.3333 | 3 | 0 |
| A | | | |
| A | 10.0000 | 3 | 100 |
| A | | | |
| A | 10.0000 | 3 | 1000 |
| A | | | |
| A | 10.0000 | 3 | 200 |
| A | | | |
| A | 10.0000 | 3 | 500 |

Eggshell Thickness

Analysis of CRD

The GLM Procedure

Dependent Variable: eshT

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 0.00051960 | 0.00012990 | 0.44 | 0.7789 |
| Error | 10 | 0.00296800 | 0.00029680 | | |
| Corrected Total | 14 | 0.00348760 | | | |

| R-Square | Coeff Var | Root MSE | eshT Mean |
|---|---|---|---|
| 0.148985 | 5.148800 | 0.017228 | 0.334600 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.00051960 | 0.00012990 | 0.44 | 0.7789 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 0.00051960 | 0.00012990 | 0.44 | 0.7789 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for eshT

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 0.000297 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 0.0313 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 0.34133 | 3 | 500 |
| A | | | |
| A | 0.33933 | 3 | 100 |
| A | | | |
| A | 0.33700 | 3 | 200 |
| A | | | |
| A | 0.32900 | 3 | 0 |
| A | | | |
| A | 0.32633 | 3 | 1000 |

Albumen Weight

Analysis of CRD

The GLM Procedure

Dependent Variable: Albwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 14.26666667 | 3.56666667 | 0.65 | 0.6383 |
| Error | 10 | 54.66666667 | 5.46666667 | | |
| Corrected Total | 14 | 68.93333333 | | | |

| R-Square | CoeffVar | Root MSE | Albwt Mean |
|---|---|---|---|
| 0.206963 | 4.981727 | 2.338090 | 46.93333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 14.26666667 | 3.56666667 | 0.65 | 0.6383 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 14.26666667 | 3.56666667 | 0.65 | 0.6383 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for Albwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 5.466667 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 4.2536 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 48.667 | 3 | 200 |
| A | | | |
| A | 47.000 | 3 | 100 |
| A | | | |
| A | 46.667 | 3 | 0 |
| A | | | |
| A | 46.667 | 3 | 1000 |
| A | | | |
| A | 45.667 | 3 | 500 |

Yolk Weight

Analysis of CRD

The GLM Procedure

Dependent Variable: yolkwt

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 1.06666667 | 0.26666667 | 0.57 | 0.6896 |
| Error | 10 | 4.66666667 | 0.46666667 | | |
| Corrected Total | 14 | 5.73333333 | | | |

| R-Square | CoeffVar | Root MSE | yolkwt Mean |
|---|---|---|---|
| 0.186047 | 3.497253 | 0.683130 | 19.53333 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 1.06666667 | 0.26666667 | 0.57 | 0.6896 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 1.06666667 | 0.26666667 | 0.57 | 0.6896 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for yolkwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 0.466667 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 1.2428 |

Means with the same letter are not significantly different.

| t Grouping | Mean | N | Enz |
|---|---|---|---|
| A | 20.0000 | 3 | 500 |
| A | | | |
| A | 19.6667 | 3 | 0 |
| A | | | |
| A | 19.3333 | 3 | 100 |
| A | | | |
| A | 19.3333 | 3 | 200 |
| A | | | |
| A | 19.3333 | 3 | 1000 |

Egg Mass

Analysis of CRD

The GLM Procedure

Dependent Variable: Eggmass

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 4 | 10588922.00 | 2647230.50 | 3.91 | 0.0365 |
| Error | 10 | 6766547.33 | 676654.73 | | |
| Corrected Total | 14 | 17355469.33 | | | |

| R-Square | Coeff Var | Root MSE | Eggmass Mean |
|---|---|---|---|
| 0.610120 | 15.78666 | 822.5903 | 5210.667 |

| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 10588922.00 | 2647230.50 | 3.91 | 0.0365 |

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Enz | 4 | 10588922.00 | 2647230.50 | 3.91 | 0.0365 |

Analysis of CRD

The GLM Procedure

t Tests (LSD) for Eggmass

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

| | |
|---|---|
| Alpha | 0.05 |
| Error Degrees of Freedom | 10 |
| Error Mean Square | 676654.7 |
| Critical Value of t | 2.22814 |
| Least Significant Difference | 1496.5 |

Means with the same letter are not significantly different.

| t Grouping | | | Mean | N | Enz |
|---|---|---|---|---|---|
| | A | | 6503.3 | 3 | 1000 |
| | A | | | | |
| B | A | | 5675.7 | 3 | 0 |
| B | A | | | | |
| B | A | C | 5022.3 | 3 | 500 |
| B | | C | | | |
| B | | C | 4859.3 | 3 | 100 |
| | | C | | | |
| | | C | 3992.7 | 3 | 200 |

**جامعة النجاح الوطنية**

**كلية الدراسات العليا**

# تأثير إضافة النواتج المجففة لتقطير الحبوب بالسوائل ومستحضرات الانزيمات على أداء دجاج البيض

**اعداد**

**ريم فتحي توفيق مصطفى**

**اشراف**

**د. معن سمارة**

**قدمت هذه الاطروحة استكمالا لمتطلبات درجة الماجستير في الإنتاج الحيواني بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس فلسطين.**

**2013**

# تأثير إضافة النواتج المجففة لتقطير الحبوب بالسوائل ومستحضرات الانزيمات على أداء دجاج البيض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## الملخص


أجريت تجربة لتقييم إضافة النواتج المجففة لتقطير الحبوب بالسوائل (DDGS) واضافة مستحضرات الانزيمات (Avizyme 1505) في علف الدجاج البياض التجاري. حيث تم استخدام 300 دجاجة بياضة من نوع (Hy–line) في المرحلة الثانية من الانتاج بعد التصويم وبعمر 69 أسبوعاً حيث تم توزيعها بشكل عشوائي وحسب (2x4 factorial arrangement)، حيث استخدمت المتغيرات (DDGS) بديلا عن الذرة وفول الصويا وكانت النسبة على مستويين (0 أو 15 ٪) وAvizyme 1505 على أربعة مستويات (0، 0.10، 0.15، 0.20 كجم/ طن). تم تقييم أداء الدجاج البياض وجودة البيض. أظهرت النتائج أن وزن الجسم وإنتاج البيض، وزن البيضة، وكتلة البيض لم تتأثر كثيرا بإضافة كلا منDDGS، Avizyme، أو التداخل بينهما. وخلصت نتائج هذه التجربة الى أن مستويات DDGS ومستحضرات الانزيمات لم تؤثر بشكل كبير على وزن الجسم وإنتاج البيض وخصائص البيض للدجاج البياض في الدورة الثانية من الانتاج. كما تم أجراء تجربة أخرى لتقييم فعالية استخدام مستحضرات الانزيمات (Avizyme 1505) في الاعلاف التجارية للدجاج البياض بمستويات متفاوتة (0، 0.100، 0.200، 0.500، 1.000 كجم / طن).

تم استخدام 150 دجاجة بياضة من نوع (Hy–line) في المرحلة الثانية من الانتاج وعلى عمر 73 أسبوع حيث تم توزيعها بشكل عشوائي. حيث تم تقييم أداء الدجاج البياض وجودة البيض. وأشارت نتائج الدراسة ان اضافة Avizyme في المستوى الموصى به تجاريا أو مستويات أعلى من ذلك لم يؤثر بشكل كبير على أي من معايير الأداء.