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Improving Opportunities for Small Flock Egg Production and Proper Evaluation of Glucanase for Commercial Poultry

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Improving Opportunities for Small Flock Egg Production and Proper Evaluation of Glucanase for Commercial Poultry

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**Thesis submitted to the Davis College of Agriculture, Natural Resources, and Design at
West Virginia University**

in partial fulfillment of the requirements for the degree of

Master of Science in Nutrition and Food Science

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ABSTRACT

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Angela Elsie Lamp

Consumers of pastured hen eggs have justified paying an associated premium price because they perceive animal welfare, sustainability, and nutrition are enhanced compared to conventionally produced eggs. The objective of Study 1 (Chapter 2) was to implement practical management strategies to increase eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) composition of eggs (Experiment 1) and to determine if the same diet formulation would produce an enhanced EPA and DHA egg composition and effect hen health when pasture access and hen breed varied (Experiment 2). For Experiment 1, four dietary treatments were utilized: 1) Basal, 2) Basal + 0.5% Sardine Oil, 3) Basal + 1% Marine Oil, and 4) Basal + 1% Flaxseed Oil. Hens fed Basal + 1% Sardine Oil produced eggs with the greatest concentration of EPA and DHA (approximately 200mg per egg). Aroma and flavor attributes determined by a taste panel did not demonstrate a dislike for pastured or EPA/DHA eggs. Experiment 2 utilized a split-plot design with housing as the whole plot unit (pasture or conventional without pasture) and a factorial arrangement of treatments applied to subplot pens (2 Breed (124 Single-Comb-White Leghorn SCWL or 124 Red Star (**RS**)) X 2 Diet (1% Sardine Oil (**Sardine**) or 1% soybean oil (**Basal**)). Egg EPA content was affected by a House X Diet interaction, demonstrating that hens fed Sardine had elevated EPA; however, the increase was greater when hens were conventionally housed without pasture. Egg DHA content was affected by Diet, showing increased DHA when hens were fed Sardine compared to Basal. These data show that egg EPA and DHA content can be influenced by both diet and housing system as defined by pasture access. In Study 2 (Chapter 3), barley based diets were fed to Cobb x Cobb 500 broilers. Dietary treatments varied in glucanase doses (125 – 2000U/kg of feed), glucanase enzyme type (GA and GB), and degree of processing (unprocessed mash and ground pellet). Inclusion of GA decreased gut viscosity (GV) and increased weight gain for ground pelleted diets, but not unprocessed mash diets. For ground pellets, GA dosed at 1000 U/kg of feed was superior to the negative control (150 kcal/kg energy decrease) and indistinguishable from the positive control for ending bird weight and weight gain. These benefits were not observed for GB, perhaps in part due to a 50% decrease in activity post pelleting. Evaluations of glucanase should go beyond in vitro activity and include live bird performance using feed that has undergone pelleting.

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KEY

CHAPTER 1

1. Polyunsaturated Fatty Acids – PUFAs
2. Eicosapentaenoic Acid – EPA
3. Docosahexaenoic Acid – DHA
4. Alpha-linolenic Acid – ALA
5. National Health and Nutrition Examination Survey – NHANES
6. Nonstarch Polysaccharide – NSP

CHAPTER 2

1. Eicosapentaenoic Acid – EPA
2. Docosahexaenoic Acid – DHA
3. Hy-line W-36 Single-Comb-White Leghorn Hen – SCWL
4. Red Star Hen – RS
5. Alpha-linolenic Acid – ALA
6. Feed Intake – FI
7. Feed Conversion Ratio – FCR
8. Alkaline Phosphatase – ALP
9. Gamma-glutamyl Transpeptidase – GGT
10. Phosphorous – PHOS
11. Albumin – ALB
12. Uric Acid – URIC
13. Aspartate Aminotransferase – AST
14. Alanine Aminotransferase – ALT
15. Glucose – Gluc
16. Cholesterol – Cholest
17. Total Protein – TRPO
18. Amylase – AMY
19. Urea Nitrogen – BUN
20. Creatine Kinase – CK
21. Albumin/Globulin Ratio – ALB/GLOB
22. Globulins – GLOB
23. Urea Nitrogen/Creatinine Ratio – BUN/CREA
24. 1% Sardine Oil – Sardine
25. 1% Soybean Oil – Basal

CHAPTER 3

1. Glucanase – Gluc
2. Digesta Viscosity – DV
3. Glucanase A – GA
4. Glucanase B – GB
5. Feed Intake – FI
6. Live Weight Gain – LWG
7. Positive Control – PC
8. Negative Control – NC
9. Hot Pellet Temperature – HPT
10. Pellet Durability Index – PDI
11. Dinitrosalicylic Acid – DNS
12. Feed Conversion Ratio – FCR

CHAPTER 1: LITERATURE REVIEW

1. SMALL SCALE POULTRY PRODUCTION PHILOSOPHY

Small scale, pastured egg production requires increased labor, land, and feed resources, thus a premium price must be obtained for eggs from these producers. Consumers justify spending more for these eggs because they perceive animal welfare and nutrition are enhanced compared to conventionally produced eggs [1]. Small scale egg producers would undoubtedly benefit from use of production strategies that alter the nutritional quality of pasture eggs relative to most conventionally produced eggs.

2. Ω -3 POLYUNSATURATED FATTY ACIDS

The ω -3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of human coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer [2 – 7]. Fatty acids typically have an even number of carbon atoms, in the range of 16-26. Fatty acids with only single bonds between adjacent carbon atoms are referred to as “saturated”; whereas, those with at least one carbon, carbon double bond are called “unsaturated”. The polyunsaturated fatty acids (**PUFAs**) have two or more double bonds, and they are named according to the position of these bonds and the total chain length [8]. The term “ ω -3” indicates that, counting from the methyl (CH_3) end of the molecule, the first double bond is located between the third and fourth carbons. As the degree of unsaturation in fatty acids increases, the melting point decreases [8]. The major ω -3 PUFAs important in human health include the essential fatty acids EPA, DHA, and ALA [8].

Alpha-linolenic acid (**ALA**) is the 18-carbon, 3-double bond ($\text{C}_{18:3\omega-3}$) precursor to eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), the latter 2 being the

predominant ω -3 fatty acids in fish oils. Alpha-linolenic acid is found in certain plant oils, most notably flaxseed oil (where it constitutes ~50% of total fatty acids) and in canola oil (~9%), unhydrogenated soybean (salad dressing) oil (~7%), hydrogenated soybean oil (~3%), and olive oil (~1%). According to National Health and Nutrition Examination Survey (**NHANES**) III data, consumption in the United States currently averages ~1.3 g/d [9].

Synthesis of DHA and EPA occurs in phytoplankton and animals, but not plants. Docosahexaenoic acid and EPA are absent from all vegetable fats and oils, including nuts, grains, and seeds. These fatty acids are also very low in ruminant fats, including milk and dairy products [10]. The richest dietary sources are fish and sea foods; and poultry and eggs provide lower, but important, sources of EPA and DHA [11]. Also, DHA is the most abundant ω -3 fatty acid in the mammalian brain. Docosahexaenoic acid levels in the brain membrane lipids increase with development and decrease with aging [12, 13, 14]. Mammals obtain DHA either as DHA itself or the precursor ALA [10].

The major ω -3 PUFAs important in human health include the essential fatty acids, ALA (18:3 ω -3), EPA (20:5 ω -3), and DHA (22:6 ω -3). Plant oils such as flaxseed, soybean, and canola are rich sources of ALA [15], while marine oils have substantial levels of both EPA and DHA [11]. All three PUFAs have distinct biological effects. In human nutrition, ALA is needed in the diet in order to synthesize longer chain PUFAs such as EPA and DHA [15]. However, the conversion efficiency of ALA to EPA and DHA is, at best, 5% in men and slightly higher in women [16]. Eicosapentaenoic acid and DHA are converted to eicosanoids and docosanoids which play an important role in regulating many biological functions: blood pressure, platelet aggregation, blood clotting, blood lipid profiles, and immune and inflammation responses during injury [17].

Health Benefits

Consumption of the ω -3 PUFAs ALA, EPA, and DHA can provide health benefits such as improvement of cognitive function, decrease in inflammatory joint pain, and improvement of the cardiovascular system [8]. A large number of clinical trials have been conducted on the effects of DHA supplementation in infants fed formula; these findings have had varied results.

Regardless of the absence of differences between placebo and DHA intervention groups, a positive association between the infants' DHA status and neurodevelopmental outcome has been shown in several studies [18, 19, 20]. Some longer-term follow-up studies are also emerging to suggest positive effects of early enhanced DHA nutrition on mental and motor skill development when measured in early childhood [21, 22]. However, some scientists find no advantage of enhanced DHA nutrition on mental and motor skill development in infants [23, 24]. Recently, attention has turned to DHA supplementation of pregnant and lactating women, again with most studies reporting no advantages to infant development during the first year after birth [18, 19, 20, 24, 25, 26].

Consuming ω -3 PUFAs can also decrease inflammatory joint pain. Excessive or inappropriate inflammation contributes to a range of acute and chronic human diseases and is characterized by the production of inflammatory cytokines, arachidonic acid– derived eicosanoids (prostaglandins, thromboxanes, leukotrienes, and other oxidized derivatives), other inflammatory agents (eg, reactive oxygen species), and adhesion molecules. At sufficiently high intakes, long-chain ω -3 PUFAs decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species and the expression of adhesion molecules [27]. Long-chain ω -3 PUFAs act directly by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism. Polyunsaturated fatty acids also act indirectly by altering the

expression of inflammatory genes through effects on transcription factor activation. Evidence of their clinical efficacy is reasonably strong in rheumatoid arthritis; but, it is weak in inflammatory bowel diseases and asthma. More, better designed, and larger trials are required to assess the therapeutic potential of ω -3 PUFAs in inflammatory diseases [27].

A third health benefit to adding ω -3 PUFAs to the diet is that it improves the cardiovascular system. Bucher and others [28] conducted a meta-analysis of 11 randomized-controlled trials which involved a total of 7,951 patients in the intervention groups and employed supplementation levels of 0.3-6.0 g day⁻¹ for EPA, and 0.6-3.7 g day⁻¹ for DHA. The meta-analysis concluded that ω -3 PUFAs could reduce overall mortality, mortality because of myocardial infarction and sudden death in patients with coronary heart disease [28]. The GISSI Intervenzione study [29] is the largest ω – 3 trial conducted involving 11,324 subjects who had survived an acute myocardium infraction. Subjects, who were followed for 3.5 years, were assigned to one of four groups: 1) 0.88 g day⁻¹ omea-3 PUFA (1:2, EPA:DHA) alone; 2) ω -3 PUFA + 300 mg day⁻¹ vitamin E; 3) vitamin E alone; 4) no treatment. The subjects given ω -3 PUFA showed a significant reduction in cardiac events. Inclusion of vitamin E offered no additional protection [29].

The incorporation of these oils (marine and soybean) into laying hen diets and the subsequent deposition into eggs may better justify egg premiums due to these fatty acids being associated with a plethora of health benefits [2 – 7]. Past research has indicated a consumer driven market for the increased production of functional foods [30, 31] and surveys have demonstrated that consumers have a growing interest surrounding the production of ω -3 enriched foods as dietary alternatives to the consumption of fish [32].

Functional Food

The incorporation of marine and soybean oil into laying hen diets and the subsequent deposition into eggs may better justify egg premiums due to these fatty acids being associated with a multitude of health benefits. The concept of healthy food additives has come from Japan in the 1970's with the term "functional foods" appearing in 1984 [33]. The Food and Nutrition Board of the National Academy of Sciences defines a functional food as one that encompasses potentially healthy products providing health benefit beyond that of traditional nutrients it contains [34]. This is in agreement with data from the recent USA study from written questionnaires completed by 2,074 qualified respondents in 1998 indicating that most shoppers believe foods can offer benefits that reach beyond basic nutrition to functional nutrition for disease prevention and health enhancement [32]. Today, functional foods receive extensive attention [30, 31] and represent one of the fastest growing divisions of the world food industry [33]. For example, dairy products and other processed foods, including mayonnaise, margarine, dressings containing DHA [35] as well as ω -3 enriched eggs [36, 37] are already on the market in different countries. In the USA, annual sales of functional food products comprise around \$50 billion [33].

Commercial table eggs contain a high proportion of ω -6 PUFA (mainly 18:2 ω -6) but are a poor source of ω -3 fatty acids. Attempts to produce eggs high in ω -3 PUFAs can be divided into two groups. 1) The simplest way is to produce an egg enriched in ALA [36], which is a precursor of DHA and is also considered to have a protective effect against fatal ischemic heart disease [38, 39]. To incorporate this ALA into the hen's diet flaxseeds, linseeds or their corresponding oils are usually added; as a result the egg's yolk is enriched with ALA and the level of DHA is also enhanced [40]. 2) The second route to enhancing levels of ω -3 in the egg is

by including pre-formed DHA in the hen's diet, usually in the form of fish (menhaden, herring or tuna) oil [37]. However, this may be associated with a pronounced fishy taste in the egg yolk.

3. BARLEY AND BARLEY USE IN POULTRY FEED

Barley use in poultry diets has been traditionally restricted in the United States due to its low energy value and subsequent sticky droppings, impairment of broiler performance, and decrease of digestion and absorption of nutrients [41 - 44]. However, today barley is being used more frequently as a feed component because of the better knowledge of its chemical composition and the remarkable progress in biotechnological production of commercial enzymes [45, 46]

The mixed linked 1, 3:1, 4- β -glucans are considered the source of all the detrimental effects barley causes poultry [41, 43, 44]. The nonstarch polysaccharide (**NSP**) portion of barley protects starch, protein, and lipids; therefore, making it challenging for digestive enzymes to reach these components [47].

4. β -GLUCANASE ENZYME SUPPLEMENTATION

The addition of a glucanase enzyme to a barley based broiler diet can provide several benefits. The enzyme can improve the efficiency of feed utilization, contribute to a better use of low cost feed ingredients [48, 49], reduce sticky droppings [41] and intestinal viscosity [50 – 52], and improve digestion and absorption of starch, protein, and fat [53, 54]. These factors all lead to increased broiler productivity [44, 53, 55].

5. ENZYME SUPPLEMENTATION, FEED MANUFACTURE, AND THERMAL PROCESSING

Enzymatic structure is very critical when it comes to the activity of a certain enzyme. An enzyme can undergo denaturation when it is exposed to heat, certain organic solvents, or extremes of pH [56].

Pelleting and glucanase supplementation are common practices utilized prior to feeding broilers barley based diets; however, the interaction of these practices is complex. Thermal stability throughout the pelleting process is a major concern for any mixer-added enzyme [57, 58]. It has been proposed that most inactivation of mixer-added enzymes take place during conditioning, when the feed is heated with saturated steam, rather than during extrusion of feed through the pellet die [59]. However, some studies have proposed mixer-added enzyme inactivation to be associated with frictional heat and pressure in the pellet die [56, 60].

Thermal processing and glucanase supplementation have been shown to have opposing effects on GV; and if glucanase is added at the mixer, then glucanase thermal stability becomes a concern. Past research has varied in methods and results on testing the thermal stability of β -glucanase. Inborr and Bedford [61] tested β -glucanase activity in feed and found that conditioning the feed at 85°C did not reduce enzyme activity compared to 75°C; however, 95°C conditioning caused significant inactivation. Esteve-Garcia and others [57] tested the effects of pelleting diets supplemented with β -glucanase at temperatures around 80°C and found that the enzymes maintained over 80% of their activity. Conversely, Almirall and others [62] incubated β -glucanase in solution at 70°C, 80°C, and 100°C and found that activity was reduced to 65, 20, and 0% respectively [57]. Viveros and others [63] autoclaved β -glucanase at 50, 70, and 90°C

and due to the increase of growth in the birds fed diets containing enzyme, no inactivated took place.

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CHAPTER 2: The Effect of Pasture Access, Breed, and Diet on Laying Hen Health, Performance, Consumer Acceptability of Eggs, and EPA and DHA Content of Eggs

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SUMMARY

The objective of this study was to implement practical management strategies to increase eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) composition of eggs (Experiment 1) and to determine if the same diet formulation would produce an enhanced EPA and DHA egg composition and effect hen health when pasture access and hen breed varied (Experiment 2). For Experiment 1, 300 Hy-line W-36 Single-Comb-White Leghorn hens (**SCWL**) were weighed and allocated 15 hens per quadrant of each of five mobile poultry houses that provided pasture access. Four dietary treatments were utilized: 1) Basal, 2) Basal + 0.5% Sardine Oil, 3) Basal + 1% Sardine Oil, and 4) Basal + 1% Flaxseed Oil. Hens fed Basal + 1% Sardine Oil produced eggs with the greatest concentration of EPA and DHA (approximately 200mg / egg, $P < 0.0001$). Aroma and flavor attributes determined by a taste panel did not indicate aversion to the pastured or EPA/DHA eggs. Experiment 2 utilized the same mobile poultry houses and raised wire conventional hen cages to compare the basal diet and basal + 1% Sardine diet. This study utilized a split-plot design with housing as the whole plot unit (pasture or conventional without pasture) and a factorial arrangement of treatments applied to subplot pens (2 Breed (124 SCWL or 124 Red Star (**RS**)) X 2 Diet (1% Sardine Oil (**Sardine**) or 1% soybean oil (**Basal**)). Egg EPA content was affected by a House X Diet interaction demonstrating that hens fed Sardine had elevated EPA; however, the increase was greater when hens were conventionally housed without pasture ($P < 0.0001$). Egg DHA content was affected by Diet, showing increased DHA when hens were fed Sardine compared to Basal ($P < 0.0001$). Aspartate Amino Transferase (**AST**) activity, was affected by a House X Breed interaction describing that the SCWL had elevated AST activity when housed conventionally without pasture (< 0.0001). However, all hens displayed a healthy serum AST activity. These data show

that egg EPA and DHA content can be influenced by both diet and housing system as defined by pasture access.

Keywords: Sardine Oil, EPA, DHA, pasture access

DESCRIPTION OF PROBLEM

Small scale, pastured egg production requires increased labor, land, and feed resources, consequently requiring a premium market price. Consumers justify spending more for these eggs because they perceive animal welfare and nutrition are enhanced compared to conventionally produced eggs. These perceptions are often unfounded. Small scale egg producers may benefit from use of production strategies that alter the nutritional quality of pasture eggs relative to most conventionally produced eggs.

The major ω -3 polyunsaturated fatty acids (**PUFAs**) important in human health include the essential fatty acid, alpha-linolenic acid (**ALA**, 18:3 ω -3), eicosapentaenoic acid (EPA, 20:5 ω -3), and docosahexaenoic acid (DHA, 22:6 ω -3). Plant oils such as flaxseed, soybean, and canola are rich sources of ALA, while fish and marine oils have substantial levels of both EPA and DHA [1]. All three PUFAs have distinct biological effects. In human nutrition, ALA is needed in the diet in order to synthesize longer chain PUFAs such as EPA and DHA. However, the conversion efficiency of ALA to EPA and DHA is, at best, 5% in men and slightly higher in women [2]. Both EPA and DHA are converted to eicosanoids and docosanoids that help regulate many biological functions such as: blood pressure, platelet aggregation, blood clotting, blood lipid profiles, and immune and inflammation responses during injury [3]. Consumption of the ω -3 PUFAs ALA, EPA, and DHA can provide human health benefits such as improvement of cognitive function, decrease in inflammatory joint pain, and improvement of the cardiovascular

system. A positive association between infants' DHA status and neurodevelopmental outcome has been shown in several studies [4, 5, 6]. At sufficiently high intakes, long-chain ω -3 PUFAs decrease the production of inflammatory eicosanoids, cytokines, and reactive oxygen species and the expression of adhesion molecules [7]. Polyunsaturated fatty acids also act indirectly by altering the expression of inflammatory genes through effects on transcription factor activation [7]. The GISSI Intervenzione study showed a significant reduction in cardiac events for subjects given ω -3 PUFAs [8].

The incorporation of these fatty acids into laying hen diets and the subsequent deposition into eggs may better justify egg premiums due to the aforementioned functions and associated health benefits. The Food and Nutrition Board of the National Academy of Sciences defines a functional food as one that encompasses potentially healthy products providing health benefit beyond that of traditional nutrients it contains [9]. This is in agreement with the data of the recent USA study from written questionnaires completed by 2,074 qualified respondents in 1998 indicating that most shoppers believe foods can offer benefits that reach beyond basic nutrition to functional nutrition for disease prevention and health enhancement [10]. Today, functional foods receive extensive attention [11, 12] and represent one of the fastest growing divisions of the world food industry [13]. Consumers have also shown interest in purchasing eggs produced by alternative management practices as opposed to conventional production (i.e. cage free, pastured, and free range) [14]. Therefore, the objective of the current study was to assess the effects of marine and flaxseed oil inclusion in diets for pastured laying flocks on hen performance, health, and EPA and DHA content of eggs.

MATERIALS AND METHODS

Both experiments housed laying hens in five 3.05 x 3.05 m (10 x 10 ft) mobile poultry houses. Each mobile house was divided into four 1.52 x 1.52 m (5 x 5 ft) pens, providing a total of 20 pens. Each pen was equipped with nipple drinkers [15], a feed hopper [16], six nesting boxes, two doors for outside access as described by Rack and others [17], and netting in order to keep hens confined to each of their respective treatments inside the house. The doors of the houses allowed access to a fenced and netted pasture of 6.40 x 7.62 m (21 x 25 ft). Pastures were equipped with a 26 liter water fount [16]. After two weeks, the hens were moved to an adjacent, identical pasture in order to provide fresh pasture. During daylight hours, hens were given outdoor access and were free to move in and out of the houses. Predation was addressed by surrounding the 5-house production system with electric fence [18], and by locking birds inside houses each night. Hens were exposed to a photoperiod of 14 hours; 4 hours of supplemental lighting each night was provided by a 60 watt incandescent light bulb per house charged by a battery and solar panel. Feed and water were supplied for *ad libitum* consumption. Hens within each pen were weighed collectively on D1. A colored leg band corresponding to treatment was placed on each hen in order to ensure birds were maintained within their respective treatment groups. Eggs were collected and counted daily. Eggs with cracked shells and/or soft shells were considered losses and not used to calculate egg production data. Eggs were collected the final two days of each study and stored at 4.4°C for two weeks prior to analysis to mimic commercial storage times. Four eggs from each diet (within each replicate) were hand separated to remove egg whites. Yolks were lyophilized for 48 hours, ground, and analyzed for fatty acid content [19].

Experiment 1

Experiment 1 was conducted in five mobile poultry houses that provided pasture access and followed management described above. Three hundred, 20-week old SCWL [20] hens were randomly allocated 15 birds per pen and provided one of four randomly assigned diets: 1) Basal; 2) Basal + 0.5% Sardine Oil; 3) Basal + 1% Sardine Oil; and 4) Basal + 1% Flaxseed Oil (Table 1). The basal diet contained a 1% soybean oil inclusion. For the other three diets, the soybean oil was either partially replaced with 0.5% Sardine oil, or completely replaced with 1% sardine oil, or 1% flaxseed oil. The sardine oil was analyzed for its fatty acid profile [19]. The sardine oil contained 17% EPA and 11% DHA and was stabilized with vitamin E [21]. The Basal diet was mixed without oil (68 kg allotments), and when needed, the respective oil was added to the diet prior to feeding. The experimental period spanned 26 days (October through November), and during this time all hens were provided one of the experimental diets as well as pasture. Data was collected to obtain starting and ending bird weight, bird feed intake (**FI**), bird intake per day, feed:egg, and percent production. Commercially available ω -3 brand eggs [22] and conventionally produced store-brand eggs [23] were used as controls to obtain comparative descriptive data for egg fatty analysis [19].

Sensory Analysis. On the final two days of the experiment, twenty-five eggs from each diet and control eggs were pooled for cooked egg evaluation. Eggs from each diet were hand-cracked, blended with a wire whisk, and thoroughly cooked as samples were needed. Cooked eggs were portioned into 57, one gram soufflé cups, fitted with lids, and designated a random three digit code [24]. The coded soufflé cups were then stored in a warming oven for no more than 45 minutes to maintain an internal temperature of 140°F until testing occurred. Fifty-seven panelists received the scrambled samples and evaluated them on aroma, flavor, texture, visual

liking, and overall liking using a 9-point hedonic scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely [25]. Water and unsalted crackers were provided for panelists to rinse their mouths between each sample. The panelists were also presented with six coded, hardboiled egg halves [26] that were visually evaluated using a 9-point hedonic scale consisting of color and yellowness of the egg yolk.

Colorimetry. Scrambled egg samples from each diet were randomly selected and instrumental color was determined with a CR-300 Minolta Chroma Meter [27] that was calibrated by using a standard white calibration plate. Color measurements were taken on each sample and were expressed in terms of CIE values for lightness (L^*), redness (a^*), and yellowness (b^*) [27].

Experiment 2

Due to the results from the sensory panel and fatty acid analyses from Experiment 1, two diets were chosen to be tested in Experiment 2: Basal (Basal); and Basal + 1% Sardine Oil (Sardine). This experiment used the same mobile poultry houses as in Experiment 1, as well as a raised wire hen cage system without pasture. The same SCWL hens (now 48 weeks of age) utilized in Experiment 1 were used in this experiment, as well as an additional breed (RS; 48 weeks of age) [28]. Sardine oil was obtained from the same source [21] and contained the same DHA and EPA as Experiment 1. Diets were prepared similarly to that described in Experiment 1. At the farm providing pasture, one hundred SCWL and one hundred RS hens were randomly allocated with 10 birds per pen to one of the two diets. Each one of the five houses had 20 SCWL and 20 RS hens total. Hens kept at the conventionally without pasture access were housed in twenty-four 0.53 x 0.38 x 0.51 m [1.73 x 1.25 x 1.67 ft] raised wire cages equipped

with nipple drinkers and a feed trough. Twenty-four SCWL and twenty-four RS hens were randomly allocated 2 birds per pen to one of the two experimental diets. The experimental period spanned 40 days (June through July), and during this time measured variables included: beginning and ending bird weight, FI, feed conversion ratio (**FCR**) based on total egg weight, FCR based on per dozen of eggs laid, and percent lay.

Blood Analyses. To determine bird health, on d 41 and 42, ten hens from each breed, dietary treatment, and housing system (80 hens total) were stunned with electricity and exsanguinated via the jugular vein. Blood from each bird was collected in a 25 mL BD Falcon tube [29] and immediately placed on ice and centrifuged at 1,500g for 10 min at 4°C so that non-fasting serum could be collected in a 1 mL microcentrifuge tube [29] and stored at -80°C until analysis. Serum parameters were determined by Vet-16 rotor colorimetric assay and measured using a Hemagen Analyst automated spectrophotometer [30]. General clinical health measurements included: alkaline phosphatase (**ALP**), gamma-glutamyl transpeptidase (**GGT**), phosphorous (**PHOS**), albumin (**ALB**), uric acid (**URIC**), total protein (**TRPO**), aspartate aminotransferase (**AST**), Alanine Aminotransferase (**ALT**), amylase (**AMY**), glucose (**Gluc**), cholesterol (**Cholest**), urea nitrogen (**BUN**), calcium, creatine kinase (**CK**), creatinine, and total bilirubin; and calculates for albumin/globulin ratio (**ALBGLOB**), globulins (**GLOB**), and urea nitrogen/creatinine ratio (**BUN/CREA**). Approximately 90 µL of serum was placed in the rotor and the rotor was then placed into the Hemagen Analyst for analysis.

Statistical Analysis

Experiment 1. Hen performance, egg fatty acid analysis, and sensory analysis variables were analyzed using a randomized complete block design. The experimental unit consisted of one pen of 15 laying hens. Diet means were further explored using Fisher's least significant

difference test. All data were statistically analyzed using the GLM procedure of Statistical Analysis System [31]. Alpha was designated as 0.05, and letter superscripts were used to denote differences among diet means.

Experiment 2. A housing X breed X treatment factorial split plot design was used to explore main effects and interactions of all treatments on performance, fatty acid analysis, and serum chemistry data. Housing was considered the whole plot unit while diet and breed were considered main effects. A randomized complete block design was utilized with one pen of either ten RS or SCWL hens as the experimental unit for hens housed with pasture. For hens housed conventionally without pasture, one pen of either two RS or SCWL hens was the experimental unit. All data were statistically analyzed using the GLM procedure of Statistical Analysis System [31]. Alpha was designated as 0.05, and letter superscripts were used to denote differences among treatment means.

RESULTS AND DISCUSSION

Experiment 1

Hen Performance. Beginning bird weight, ending bird weight, bird feed intake (FI), bird intake per day, feed:egg, and percent production were not affected by dietary treatment ($P > 0.05$, Table 2). This is consistent with results observed by Gonzalez-Esquerria and coauthors [32] where fish oil inclusion had no effect on hen weight or egg production during a 19 to 55wk study period.

Fatty Acid Analysis. Table 3 demonstrates the ALA (18:3 ω -3), EPA (20:5 ω -3), and DHA (22:6 ω -3) fatty acid composition from representative samples of each treatment within each replicate. Data was analyzed on a percentage of sample basis. There were no significant

differences among pastured hen egg percent ALA ($P > 0.05$); however, the pastured hen eggs had a numerically higher percentage of ALA than the descriptive data obtained from the control eggs. Gonzalez-Esquerria and coauthors [32] observed no difference in ALA content when treatments varied by either 2% regular fish oil or 2% deodorized fish oil. Hens fed Basal + 1% Sardine Oil demonstrated the highest percentage of EPA, while hens fed Basal and Basal + 1% Flaxseed Oil demonstrated the lowest. Hens fed Basal + 0.5% Sardine Oil demonstrated an intermediate level of EPA percentage ($P < 0.05$), and the descriptive data obtained from the control eggs contained no detectable EPA. Hens fed Basal + 1% Sardine Oil provided eggs that had the highest percentage of DHA, while hens fed Basal produced eggs with the lowest DHA content. Hens fed Basal + 0.5% Sardine Oil and Basal + 1% Flaxseed Oil produced eggs with an intermediate percentage of DHA ($P < 0.05$). In addition, all pasture treatments had a numerically higher percentage of DHA than the descriptive data obtained from the control eggs. The increase in percentage of EPA and DHA in eggs as a result of feeding hens fish oil ($P < 0.01$) was also observed by Gonzalez-Esquerria and coauthors [32]. In our study, hens fed the Basal + 1% Sardine Oil and Basal + 1% Flaxseed Oil diets produced eggs with a higher percentage of total ω -3 fatty acids ($P < 0.05$) compared to hen fed Basal which provided eggs with the lowest percentage. All pasture diets had a numerically higher percentage of total ω -3's compared to the descriptive data obtained from control eggs. There were no significant differences observed for the variable percent ω -6 PUFAs ($P > 0.05$) among pasture treatments. However, pastured hen eggs had a numerically higher percentage of ω -6 fatty acids compared to the descriptive data obtained from the control eggs. This contrasts results by Van Elswyk and coauthors [33] where a decrease in yolk ω -6 fatty acids was observed due to fish oil inclusion in hen diets. The ω -6: ω -3 of eggs was highest for hens fed Basal, and lowest for hens fed Basal + 0.5% Sardine Oil, Basal

+ 1% Sardine Oil, and Basal + 1% Flaxseed Oil ($P < 0.05$). Numerically, the descriptive data obtained from the control eggs had almost double the ratio compared to pastured hen eggs.

An average sized egg weighs approximately 57000 mg, and the yolk constitutes about 31% of the egg weight [34]. Therefore, the weight of an average sized egg yolk is about 18000 mg. The dry matter percentage of an egg yolk is approximately 50%, meaning the dry portion of yolk is 9000 mg [34, 35]. The eggs in Experiment 1 produced by hens fed Basal + 1% Maine had an EPA and DHA content of 0.214 and 2.22% respectively. Knowing that the dry portion of yolk is 9000 mg, we can calculate that the EPA and DHA content of these eggs is 19 and 200 mg/egg respectively. Hens fed Basal + 1% Sardine produced eggs with a total EPA and DHA content of 219 mg/egg. The American Heart Association advises patients with documented coronary heart disease consume approximately 1000 mg per day of EPA and DHA [36]. Consuming two of the eggs produced by a hen fed the Basal + 1% Sardine Oil diet in this experiment could provide 40% of this recommendation.

Sensory Analysis. All sensory analysis data can be found in Table 4.

Scrambled Samples. There were no significant differences found for aroma, flavor, and visual liking for the scrambled samples ($P > 0.05$). For texture, eggs collected from hens fed Basal + 1% Flaxseed Oil and the control eggs received a higher rating, while eggs collected from hens fed Basal + 1% Sardine Oil ($P < 0.05$) had the lowest rating. Similar results were found for overall liking ($P < 0.05$). However, mean ratings did not fall into a category of dislike for any egg.

Hardboiled Samples. The Basal + 0.5% Sardine Oil eggs had the most appealing color, while the conventionally produced store-brand eggs were the least appealing in color ($P < 0.05$). Eggs produced by hens fed Basal, Basal + 1% Sardine Oil, Basal + 1% Flaxseed Oil, and the

commercially available ω -3 eggs received an intermediate rating on color. The Basal + 0.5% Sardine Oil hardboiled eggs obtained the highest rating for yellowness of yolk. The Basal + 1% Flaxseed Oil and conventionally produced store-brand eggs obtained the faintest yolks ($P < 0.05$), while hens fed Basal, Basal + 1% Sardine Oil, and commercially available ω -3 eggs produced eggs with an intermediate yellow yolk. Gonzalez-Esquerre and coauthors [32] also provided hardboiled egg halves to panelists to evaluate different characteristics of the eggs. The scientists found that most of the panelists described the sensory attributes of the eggs produced by hens fed the fish oil as “fishy.” The authors of the current study did inquire the panelist about an aftertaste, and received no comments concerning a fishy aftertaste. The current study supplemented Sardine oil at 0.5 and 1%. However, Gonzalez-Esquerre and coauthors [32] supplemented their diets with 2, 4, and 6% sardine oil which may have led to the reaction they received.

Colorimetry. All eggs produced by hens with access to pasture were the lightest, while the conventional and commercial ω – 3 eggs were darker ($P < 0.05$). There were no significant differences observed for the variable of redness among the different eggs ($P > 0.05$). The yellowness was least in the conventionally produced eggs, and greatest in all other pasture produced eggs ($P < 0.05$). The data for colorimetry can be found in Table 5.

Experiment 2.

Hen Performance. Table 6 contains the hen performance data for Experiment 2. The main effect Diet had no effect on hen performance ($P > 0.05$). Starting bird weight was significantly affected by House and Breed; hens housed conventionally without pasture were larger than hens housed with pasture access and the RS hens were larger than the SCWL ($P <$

0.05). For ending bird weight, there was a House X Breed interaction ($P = 0.05$) describing that the RS hens were larger than the SCWL hens on D40; however, the weight difference was greatest when hens were reared conventionally without pasture. On D40, the main effect Breed influenced FI per bird describing that the RS hens consumed more than the SCWL hens, 5.36 vs. 3.68 kg per bird, respectively ($P < 0.0001$). For FCR total egg weight and FCR per dozen, there was a House X Breed interaction ($P < 0.0001$) demonstrating that when hens were housed with pasture access, FCR favored SCWL hens; however, when hens were reared conventionally without pasture, FCR favored the RS hens. Percent lay also demonstrated a House X Breed interaction ($P < 0.0001$) describing that when hens were housed with pasture access, percent lay favored SCWL hens; however, when hens were housed conventionally without pasture, percent lay favored RS hens. These results were likely associated with the RS hens' propensity for foraging and larger body size that would require greater maintenance, and variations in percent lay.

Serum Chemistry. We did not obtain values for several serum measurements using the Hemagen Analyst due to values that were not provided.

Clinical measurements that were obtained can be found in Table 7. ALB and GLOB were not significantly affected by house, breed, or diet ($P > 0.05$). Still, both ALB and GLOB serum levels on average were in normal range for all hens, 1.3 – 2.8 and 1.5 – 4.1 g/dL respectively [37]. For serum ALP activity, the main effect House was significant, demonstrating that hens housed conventionally without pasture had a higher serum ALP activity than hens housed with pasture access, 464.00 vs. 415.53 U/L, respectively ($P = 0.0232$). The normal ALP serum activity in noncarnivorous birds is less than 10 U/L [38]. Our ALP value was higher than the normal value. Some medical problems that could have been the cause are

hyperparathyroidism induced fractures, egg laying, hepatic disease, enteritis, and aflatoxicosis [37]. Özbey and Esen [39] performed a study using rock partridge chicks housed using different stocking densities. Their results opposed ours; ALP activity slightly decreased as stocking density decreased. In our study, the main effect House was also significant ($P < 0.0001$) for serum PHOS and URIC levels, describing that hens housed with pasture access had a higher serum PHOS and URIC levels than hens housed conventionally without pasture. Carpenter [37] states that the normal ranges of PHOS and URIC are 6.2 – 7.9 and 2.5 – 8.1 mg/dL respectively. URIC serum values for all hens fall into the normal range. PHOS levels, on the other hand, are higher than the normal range (on average about 8.5 – 9 mg/dL). Carpenter [37] explains that this spike could be due to postprandial sampling, severe renal disease, nutritional secondary hyperparathyroidism, or hypoparathyroidism. For serum ALP, GGT, AMY, and Cholest levels, the main effect Breed was significant ($P < 0.05$) demonstrating that SCWL hens had higher serum ALP, GGT, AMY, and Cholest levels compared to RS hens. Although reference intervals have not been established, the Schubot Exotic Bird Health Center [40] considers the “normal” value of GGT to be 0 – 10 U/L. We obtained levels that were approximately 25 U/L greater than the normal range. An increase in GGT activity indicated biliary cholestasis and hyperplasia of bile ducts [41, 42]. Normal serum values for serum AMY in avians range between 100 and 600 U/L [43]. All hens demonstrated a normal serum AMY activity. The normal serum Cholest level in chicken is found to be 86 -211 mg/dL [37, 44]. Both breeds housed at either location and fed either diet had a normal serum Cholest level. The main effect Diet was significant for serum TRPO activity, demonstrating that hens fed Sardine had a higher serum TRPO activity than hens fed Basal; 6.13 vs. 5.73 g/dL respectively ($P = 0.0155$). The normal TRPO activity of a chicken is 3.3 – 5.5 g/dL [37]. We obtained values that were slightly higher than the normal

value. This could indicate that the hens could have chronic hepatopathy, malabsorption, renal disease, or neoplasia [37]. There was a House X Breed interaction ($P < 0.0001$) for the serum AST level describing that SCWL hens housed conventionally without pasture had the highest serum AST levels, 279.7 U/L. Similar values were also observed by Hrubec and coauthors [45], where they obtained a serum AST value of 219 U/L using a male single-comb white leghorn model. So and coauthors [46] observed higher serum AST levels in commercial laying hens with fatty liver hemorrhagic syndrome compared to hens without this syndrome. Our serum AST values fell in the healthy range; serum levels greater than 350 U/L are considered abnormal and are often indicative of liver, muscle, and heart damage [37, 43]. A House X Breed interaction ($P = 0.0232$) was also found for serum ALT level demonstrating that RS hens had higher serum ALT levels; however, the increase was greater for hens reared conventionally without pasture. The normal range for serum ALT has not been extensively researched, but it has been said to be 1.5 – 7.5 U/L [47]. The values we obtained were greater than the normal range. Zantop [48] states that elevated ALT activities can be caused by damage to tissues. For serum Gluc level, a House X Breed X Diet interaction ($P = 0.0313$) was significant demonstrating that hens fed Sardine had higher Gluc levels. For SCWL hens, serum Gluc levels were the highest when hens were housed conventionally without pasture; and for RS hens, levels were the highest when hens were housed with pasture access. The normal serum Gluc range for chicken is 227 – 350 mg/dL [37, 49]. All hens had a serum level within this range indicating that there were no problems with serum Gluc.

Seven out of the twelve serum parameters fell in the normal range. One more than half of the parameters exhibited signs of a healthy avian status; on the other hand, the other five parameters indicated detriment to hen health. The five parameters that were elevated past a

healthy range were ALP, PHOS, GGT, TRPO, and ALT. However, we did not observed any outward signs of discomfort or sickness nor mortalities throughout the duration of the study. Carpenter [37] states that the increase in serum ALP levels could be due to egg laying, and also explains that the increase in serum PHOS levels could be the cause of postprandial sampling. We obtained serum GGT and ALT levels greater than their normal ranges; however, the normal ranges for serum GGT and ALT have not been extensively researched [40, 47]. Carpenter [37] states that the increase in serum TROP could be caused by a simple means of utilizing a non-temperature compensated refractometer. The authors want to point out that these serum measurements and values are used as a screening method to which a veterinarian may further look into the diagnosis of health issues. Therefore, we can conclude from serum chemistry that we found neither consistent improvement nor detriment to hen health when examining housing environment, breed, and diet.

Egg Fatty Acid Analysis. The fatty acid analysis of eggs produced in Experiment 2 can be found in Table 8, data was analyzed on an mg per egg basis. For EPA content of eggs, there was a House X Diet interaction ($P = 0.0278$) describing that hens fed Sardine produced eggs with a higher EPA content; however, the increase was greater for hens reared conventionally without pasture. For DHA, the main effect Diet was significant ($P < 0.0001$), demonstrating that hens fed Sardine had a higher DHA content compared to hens fed Basal. The highest content of EPA + DHA was achieved in Sardine fed conventionally housed hens (SCWL = 132 mg/egg, RS = 141 mg/egg). There was a House X Breed interaction for ALA content of eggs ($P = 0.0002$) demonstrating that RS hens housed with pasture access produced eggs with the highest ALA content. There was also a House X Breed interaction for total ω -6 PUFAs content of eggs ($P = 0.0119$) demonstrating that SCWL hens produced eggs with a higher total ω -6 content; however,

the increase was greater for hens reared conventionally without pasture. For total ω -3 content of eggs, there was a House X Breed interaction ($P = 0.0166$) describing that the breeds differed most in total ω -3 when hens were provided pasture (RS hens with more and SCWL hens with less content of total ω -3's). There was a House X Breed X Diet interaction ($P = 0.0229$) for the ratio of ω -6: ω -3 content of eggs describing that Basal, lack of pasture, and the SCWL breed contributed to increasing the ω -6: ω -3 ratio.

The American Heart Association advises patients with coronary heart disease consume approximately 1000 mg per day of EPA and DHA [36]. Consuming two of the eggs produced by a hen fed the Basal + 1% Sardine Oil diet in this experiment could provide 25% of this recommendation.

CONCLUSIONS AND APPLICATIONS

Experiment 1

1. Hen performance variables were not affected and sensory panel data deemed the eggs produced by hens fed Basal + 1% Sardine to be acceptable.
2. Hens fed the Basal diet had the lowest EPA and DHA levels, followed by Basal + 0.5% Sardine Oil and Basal +1% Flaxseed Oil. Hens fed the Basal + 1% Sardine Oil produced eggs with the greatest concentration of EPA and DHA (219 mg/egg total).
3. The Basal + 1% Sardine eggs were superior to the conventionally produced store-brand eggs for color and yellowness of yolk for hardboiled samples. The authors of the current study did inquire the panelist about an aftertaste, and the panelists replied that there was no fishy aftertaste.

Experiment 2

1. Egg DHA content was affected by diet, showing increased DHA when hens were fed Sardine compared to Basal (123 vs. 46 mg/egg, respectively). Egg EPA content was affected by a House X Diet interaction, demonstrating that the hens fed Sardine had elevated EPA; however, the increase was greater when hens were conventionally housed without pasture.
2. Through serum chemistry measurements, we found neither consistent improvement nor detriment to hen health when examining housing environment, breed, and diet.
3. These data show that egg EPA and DHA content can be influenced by both diet and housing system as defined by pasture access.

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Table 1. Basal Diet Formulation

Ingredient	Inclusion (%)
Corn	58.76
Soybean Meal	19.56
Limestone	8.97
Corn Gluten Meal	5.00
Wheat Middlings	3.88
Defluorinated Phosphorus	2.20
Soybean or Sardine Oil ¹ or Flaxseed Oil	1.00
NB 3000 ²	0.25
Salt	0.13
Methionine	0.13
Lysine	0.05
Coban 90 ³	0.05
Phytogetic Feed Additive	0.02
Calculated Nutrients	
Metabolizable Energy (kcal/kg)	2948
Crude Protein (%)	18.22
Lysine (%)	0.88
Methionine + Cysteine (%)	0.76
Calcium (%)	4.00
Available Phosphorus (%)	0.50
Sodium (%)	0.18

¹Obtained from Jedwards International, Inc., Quincy, MA

²Vitamin-mineral premix (NB3000, Nutrablend, Neosho, MO) supplied the following per kilogram of diet: manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B₆, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B₁₂, 0.01 mg; vitamin E, 16.53 IU; vitamin D₃, 2,133 ICU; vitamin A, 7,716 IU.

³Active drug ingredient monensin sodium, 60 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN) as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria aceryulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

Table 2. Effects of oil inclusion on laying hen performance data (Experiment 1).

Treatment¹	Beginning Bird Wt.² (kg)	Ending Bird Wt.² (kg)	Bird Feed Intake (kg)	Bird Intake Per Day	Feed:Egg (kg:dozen)	Percent Production (%)
Basal	1.29	1.23	1.72	0.07	3.77	34.81
Basal + 0.5% Sardine Oil	1.28	1.23	1.54	0.06	3.24	34.63
Basal + 1% Sardine Oil	1.25	1.22	1.73	0.07	3.51	37.63
Basal + 1% Flaxseed Oil	1.24	1.26	1.67	0.06	2.68	46.58
ANOVA P-value	0.3847	0.7371	0.7894	0.7894	0.1997	0.1066

¹Treatments: Basal – corn-soybean based diet with a 1% soybean oil inclusion; Basal + 0.5% Sardine Oil – corn-soybean based diet with a 0.5% Sardine oil inclusion; Basal + 1% Sardine Oil – corn-soybean based diet with a 1% sardine oil inclusion; Basal + 1% Flaxseed Oil – corn-soybean based diet with a 1% flaxseed oil inclusion; All hens were also presented with pasture access

²Hens were weighed by pen; however, mean weight per bird is presented

Table 3. Fatty acid content found performing a fatty acid analysis on eggs collected in this experiment (Experiment 1).

Treatment¹	ALA⁴ (%)	EPA⁵ (%)	DHA⁶ (%)	ω-3 (%)	ω-6 (%)	ω-6: ω-3
Basal	0.15	0.048 ^c	1.07 ^c	2.04 ^c	20.29	10.46 ^a
Basal + 0.5% Sardine Oil	0.15	0.124 ^b	1.92 ^b	2.95 ^b	19.41	6.58 ^b
Basal + 1% Sardine Oil	0.128	0.214 ^a	2.22 ^a	3.50 ^{ab}	20.29	5.84 ^b
Basal + 1% Flaxseed Oil	0.13	0.082 ^c	1.67 ^b	3.87 ^a	20.47	5.35 ^b
ANOVA P-value	0.0791	< 0.0001	< 0.0001	0.0003	0.1785	0.0003
Fisher's LSD	---	0.0414	0.2626	0.6372	---	1.9239
Descriptive Data						
Commercially available ω-3 ²	0.06	0	0.72	1.3	11.46	8.8
Conventionally produced ³	0.05	0	0.23	0.67	11.78	17.5

^{a-c} Values within comparisons with different superscripts differ ($P \leq 0.05$)

¹Treatments: Basal – corn-soybean based diet with a 1% soybean oil inclusion; Basal + 0.5% Sardine Oil – corn-soybean based diet with a 0.5% sardine oil inclusion; Basal + 1% Sardine Oil – corn-soybean based diet with a 1% sardine oil inclusion; Basal + 1% Flaxseed Oil – corn-soybean based diet with a 1% flaxseed oil inclusion; All hens were also presented with pasture access

²Commercially available ω-3 – Eggland's Best eggs

³Conventionally produced - Kroger brand eggs

⁴ALA (alpha-linolenic acid (ALA, 18:3ω-3))

⁵EPA (eicosapentaenoic acid (EPA, 20:5ω-3))

⁶DHA (docosahexaenoic acid (DHA, 22:6ω-3))

Table 4. Sensory Analysis (Experiment 1).

Treatment ¹	Scrambled Samples					Hardboiled Samples	
	Aroma	Flavor	Texture	Visual Liking	Overall Liking	Color	Yellowness of Yolk
Basal	5.31	5.28	5.51 ^{ab}	5.65	5.33 ^{ab}	4.91 ^b	5.76 ^b
Basal + 0.5% Sardine Oil	4.91	5.53	5.64 ^{ab}	5.69	5.65 ^{ab}	7.04 ^a	6.87 ^a
Basal + 1% Sardine Oil	4.77	4.84	5.00 ^b	5.02	4.75 ^b	4.65 ^b	5.51 ^b
Basal + 1% Flaxseed Oil	5.45	5.70	6.14 ^a	5.82	6.02 ^a	4.04 ^b	4.59 ^c
Commercially available ω -3 ²	5.10	5.63	6.05 ^a	5.77	5.91 ^a	4.65 ^b	5.51 ^b
Conventionally produced ³	5.35	5.41	6.07 ^a	5.96	5.76 ^a	3.15 ^c	3.71 ^c
ANOVA P-value	0.2838	0.1499	0.0018	0.091	0.001	< 0.0001	< 0.0001

^{a-c} Values within comparisons with different superscripts differ ($P \leq 0.05$)

¹Treatments: Basal – corn-soybean based diet with a 1% soybean oil inclusion; Basal + 0.5% Sardine Oil – corn-soybean based diet with a 0.5% Sardine oil inclusion; Basal + 1% Sardine Oil – corn-soybean based diet with a 1% Sardine oil inclusion; Basal + 1% Flaxseed Oil – corn-soybean based diet with a 1% flaxseed oil inclusion; All hens were also presented with pasture access

²Commercially available ω -3 – Eggland's Best eggs

³Conventionally produced - Kroger brand eggs

⁴9-point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely [Peryam, D. R., and F. J. Pilgrim. 1957. Hedonic scale method of measuring food preferences. Food Technol. 11:9–14.].

Table 5. Colorimetry (Experiment 1).

Treatment¹	L*⁴	a*⁴	b*⁴
Basal	56.33 ^a	-3.37	43.35 ^a
Basal + 0.5% Sardine Oil	48.24 ^{ab}	-2.83	35.32 ^a
Basal + 1% Sardine Oil	50.53 ^{ab}	-3.57	34.88 ^a
Basal + 1% Flaxseed Oil	53.12 ^{ab}	-3.18	39.21 ^a
Commercially available ω -3	47.32 ^{bc}	-2.40	32.55 ^{ab}
Conventionally produced	39.42 ^c	-3.62	22.49 ^b
ANOVA P-value	0.0003	0.2	0.001
Fisher's LSD	5.3	---	7.32

^{a-c} Values within comparisons with different superscripts differ ($P \leq 0.05$)

¹Treatments: Basal – corn-soybean based diet with a 1% soybean oil inclusion; Basal + 0.5% Sardine Oil – corn-soybean based diet with a 0.5% Sardine oil inclusion; Basal + 1% Sardine Oil – corn-soybean based diet with a 1% Sardine oil inclusion; Basal + 1% Flaxseed Oil – corn-soybean based diet with a 1% flaxseed oil inclusion; All hens were also presented with pasture access

²Commercially available ω -3 – Eggland's Best eggs

³Conventionally produced - Kroger brand eggs

⁴CIE values for lightness (L*), redness (a*), and yellowness (b*). Color measurements were taken on each sample using a CR-300 Minolta Chroma Meter [Minolta Co., Ramsey, NJ]

Table 6. Effects of housing environment, breed, and diet on laying hen performance (Experiment 2).

Housing Environment	Breed	Diet	Average Starting Bird Weight ¹ (kg)	Ending Bird Weight ¹ (kg)	Feed Intake/Bird (kg)	FCR Total Egg Weight (g:g)	FCR per Dozen (kg:dozen)	Percent Lay
Marginal Means								
Pasture ²	---	---	1.58 ^b	1.62	4.60	3.03	2.37 ^a	64.00 ^b
Conventional ³	---	---	1.68 ^a	1.67	4.44	2.91	1.91 ^b	74.00 ^a
---	SCWL ⁴	---	1.37 ^b	1.32 ^b	3.68 ^b	2.66 ^b	1.82 ^b	64.00 ^b
---	RS ⁵	---	1.89 ^a	1.97 ^a	5.36 ^a	3.29 ^a	2.46 ^a	74.00 ^a
---	---	Basal ⁶	1.62	1.64	4.62	2.97	2.16	69.00
---	---	Sardine ⁷	1.64	1.65	4.41	2.97	2.11	69.00
Main Effects and Interaction Probabilities								
Effect			P > F					
House			0.0009	0.0648	0.2097	0.3682	0.0002	0.0196
Breed			< 0.0001	< 0.0001	< 0.0001	0.0007	< 0.0001	0.0245
Diet			0.4983	0.5243	0.2705	0.6104	0.9351	0.6010
House x Breed			0.2970	0.0500	0.5259	< 0.0001	< 0.0001	< 0.0001
House x Diet			0.4109	0.8745	0.7965	0.8006	0.9307	0.7667
Breed x Diet			0.1197	0.2146	0.6506	0.4475	0.6934	0.7421
House x Breed x Diet			0.4209	0.2304	0.4679	0.3136	0.4951	0.2628

^{a-c}Values within comparisons with different superscripts differ (P≤0.05)

¹Hens were weighed by pen, however, mean weight per bird is presented

²Pasture – hens housed with provided pasture access

³Conventional – hens housed conventionally without pasture

⁴SCWL (Hy-line W36 Single Comb White Leghorn hen [Hy-line])

⁵RS (Red Star hen)

⁶Basal – corn-soybean based diet with a 1% soybean oil inclusion

⁷Sardine – corn-soybean based diet with a 1% sardine oil inclusion

Table 7. Effects of housing environment, breed, and diet on serum chemistry (Experiment 2).

Housing Environment	Breed	Diet	ALP ⁷ (U/L)	GGT ⁸ (U/L)	PHOS ⁹ (mg/dL)	ALB ¹⁰ (g/dL)	URIC ¹¹ (mg/dL)	AST ¹² (U/L)	ALT ¹³ (U/L)	Gluc ¹⁴ (mg/dL)	Cholest ¹⁵ (mg/dL)	TRPO ¹⁶ (g/dL)	AMY ¹⁷ (U/L)
Marginal Means													
Pasture ¹			415.53	30.08	9.86 ^a	1.83	7.31 ^a	194.10 ^b	218.03	277.56	158.38	5.97	240.49
Conventional ²			464.00	26.88	8.02 ^b	2.10	4.89 ^b	230.60 ^a	182.50	268.70	149.37	5.89	326.06
	SCWL ³		520.09 ^a	30.75 ^a	9.22	2.18	6.06	236.59 ^a	165.69 ^b	278.05	172.86 ^a	6.09	337.91
	RS ⁴		380.50 ^b	26.20 ^b	8.66	1.75	6.13	189.18 ^b	233.54 ^a	268.23	134.51 ^b	5.77	232.00
		Basal ⁵	427.07	27.50	8.61	2.00	5.79	207.48	207.65	261.40 ^b	146.96	5.73	233.80
		Sardine ⁶	436.30	29.45	9.27	1.93	6.40	217.82	194.47	285.05 ^a	161.08	6.13	332.94
Main Effects and Interaction Probabilities													
Effect			P > F										
House			0.0232	0.1265	< 0.0001	0.2460	< 0.0001	< 0.0001	0.0983	0.2442	0.5987	0.5868	0.0902
Breed			0.0018	0.0312	0.2090	0.0617	0.8656	< 0.0001	0.0024	0.1986	0.0349	0.0514	0.0467
Diet			0.1929	0.3494	0.1398	0.7750	0.1589	0.2566	0.6016	0.0030	0.4561	0.0155	0.0533
House x Breed			0.4524	0.8473	0.0921	0.3348	0.7571	< 0.0001	0.0232	0.2562	0.9040	0.4290	0.8923
House x Diet			0.1567	0.9616	0.6255	0.3031	0.5481	0.8636	0.1206	0.6921	0.4027	0.9134	0.7230
Breed x Diet			0.0808	0.9042	0.1204	0.5101	0.3920	0.6990	0.1918	0.2574	0.3146	0.4290	0.2818
House x Breed x Diet			0.3954	0.8098	0.5788	0.5679	0.1194	0.3556	0.4077	0.0313	0.8303	0.2337	0.4498

^{a-d}. Values within comparisons with different superscripts differ (P≤0.05)

¹Pasture – hens housed with provided pasture access

²Conventional – hens housed conventionally without pasture access

³SCWL (Hy-line W36 Single Comb White Leghorn hen [Hy-line])

⁴RS (Red Star hen)

⁵Basal – corn-soybean based diet with a 1% soybean oil inclusion

⁶Sardine – corn-soybean based diet with a 1% sardine oil inclusion

⁷ALP (alkaline phosphatase)

⁸GGT (gamma-glutamyl transpeptidase))

⁹PHOS (phosphorous)

¹⁰ALB (albumin)

¹¹URIC (uric acid)

¹²AST (aspartate aminotransferase)

¹³ALT (alanine aminotransferase)

¹⁴Gluc (glucose)

¹⁵Cholest (cholesterol)

¹⁶TRPO (total protein)

¹⁷AMY (amylase)

Table 8. Effects of housing environment, breed, and diet on fatty acid content of eggs collected in this experiment (Experiment 2)

Housing Environment	Breed	Diet	Yolk Size (kg)	EPA ⁷ (mg/egg)	DHA ⁸ (mg/egg)	ALA ⁹ (mg/egg)	Total ω - 3 (mg/egg)	Total ω - 6 (mg/egg)	ω - 6: ω - 3
Marginal Means									
Pasture ¹	---	---	0.01	2.70 ^b	84.11	30.33 ^a	121.21	889.30	8.66 ^b
Conventional ²	---	---	0.01	5.51 ^a	87.07	24.51 ^b	123.10	867.44	8.95 ^a
---	SCWL ³	---	0.01	3.66	83.01	29.79 ^a	120.75	938.65 ^a	9.14 ^a
---	RS ⁴	---	0.01	4.46	87.96	25.31 ^b	123.45	821.65 ^b	8.48 ^b
---	---	Basal ⁵	0.01	0 ^b	46.23 ^b	29.81 ^a	76.04 ^b	986.41 ^a	13.08 ^a
---	---	Sardine ⁶	0.01	7.93 ^a	122.90 ^a	25.30 ^b	165.91 ^a	776.28 ^b	4.74 ^b
Main Effects and Interaction Probabilities									
Effect			P > F						
House			0.3862	0.0278	0.7498	0.0001	0.9710	0.7480	0.0422
Breed			0.2698	0.3780	0.0613	0.0008	0.3351	0.0002	0.0011
Diet			0.3333	< 0.0001	< 0.0001	0.0008	< 0.0001	< 0.0001	< 0.0001
House x Breed			0.7742	0.5187	0.0985	0.0002	0.0166	0.0119	0.0330
House x Diet			0.8712	0.0278	0.3890	0.9975	0.1423	0.2995	0.0004
Breed x Diet			0.6847	0.3780	0.0784	0.8959	0.0673	0.6935	0.1518
House x Breed x Diet			0.2825	0.5187	0.3735	0.9581	0.6670	0.6204	0.0229

^{a-e} Values within comparisons with different superscripts differ (P≤0.05)

¹Pasture – hens housed with provided pasture access

²Conventional – hens housed conventionally without pasture access

³SCWL (Hy-line W36 Single Comb White Leghorn hen [Hy-line])

⁴RS (Red Star hen)

⁵Basal – corn-soybean based diet with a 1% soybean oil inclusion

⁶Sardine – corn-soybean based diet with a 1% sardine oil inclusion

⁷EPA (eicosapentaenoic acid (20:5ω-3))

⁸DHA (docosahexaenoic acid (22:6ω-3))

⁹ALA (alpha-linolenic acid (18:3ω-3))

CHAPTER3: The Effects of Pelleting and Glucanase Supplementation in Hulled Barley Based Diets on Feed Manufacture, Broiler Performance, and Digesta Viscosity

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Primary Audience: Nutritionists, Researchers

SUMMARY

Feeding broilers barley based diets, high in beta-glucan, requires special consideration primarily due to effects on increased digesta viscosity (**DV**) and decreased nutrient digestion. Pelleting and glucanase (**gluc**) supplementation are common practices utilized prior to feeding broilers barley based diets; however, the interaction of these practices is complex. Thermal processing and gluc supplementation have been shown to have opposing effects on DV; and if gluc is added at the mixer, then gluc thermal stability becomes a concern. The study utilized a randomized complete block design with eight replications of 10 straight-run Cobb x Cobb 500 broilers. Dietary treatments varied in gluc dose (125 – 2000U/kg of feed), gluc enzyme type (GA and GB), and degree of processing (unprocessed mash and ground pellet). Broilers fed ground pellets had a greater pen feed intake (**FI**) compared to birds fed unprocessed mash diets. Inclusion of GA decreased DV and increased weight gain for ground pelleted diets, but not unprocessed mash diets. For ground pellets, GA dosed at 1000 U/kg of feed was superior to the negative control (150 kcal/kg energy decrease) and indistinguishable from the positive control for ending bird weight and weight gain. These benefits were not observed for GB, perhaps in part due to a 50% decrease in activity post pelleting. Evaluations of gluc should go beyond in vitro activity and include live bird performance using feed that has undergone pelleting.

DESCRIPTION OF PROBLEM

Barley use in poultry diets has been traditionally limited in the United States due to its low energy value and subsequent sticky droppings, impairment of broiler performance, and decrease of digestion and absorption of nutrients [1, 2]. However, today barley is being used more

frequently as a diet component because of the better knowledge of its chemical composition and the remarkable progress in biotechnological production of commercial enzymes [3, 4].

The addition of a gluc enzyme to a barley based broiler diet can provide several benefits. The enzyme can improve the efficiency of feed utilization, contribute to a better use of low cost feed ingredients [5, 6], reduce sticky droppings [1], and improve digestion and absorption of starch, protein, and fat [7, 8]. These factors all lead to increased broiler productivity.

Thermal stability throughout the pelleting process is a major concern for any mixer-added enzyme [9, 10]. It has been proposed that most inactivation of mixer-added enzymes take place during conditioning, when the feed is heated with saturated steam, rather than during extrusion of feed through the pellet die [11]. However, some studies have proposed mixer-added enzyme inactivation to be associated with frictional heat and pressure in the pellet die [12].

Past research has varied in methods and results on testing the thermal stability of β -gluc. Inborr and Bedford [13] tested thermal stability of a commercial β -gluc feed enzyme product (Avizyme SX[®]) at three levels of inclusion (0, 1, and 10 g/kg) to a barley and wheat based diet. Based on bird performance, the scientists concluded that conditioning a feed and enzyme mixture at 85°C did not reduce enzyme activity compared to 75°C; however, 95°C conditioning caused significant inactivation. Esteve-Garcia and coauthors [9] tested the effects of pelleting wheat and barley diets supplemented with β -gluc (added as 2% of the total mix) at temperatures around 80°C and found that the enzymes maintained over 80% activity. Conversely, Almirall and Esteve-Garcia [14] incubated 4000 U/mL of β -gluc obtained from *Trichoderma longibrachiatum* in solution at 70°C, 80°C, and 100°C and found that activity was reduced to 65, 20, and 0% respectively of original dose activity [9].

The objective of this study was to evaluate dosages of gluc preparations added at the mixer to barley based diets (45% of diet formulation) in unprocessed mash and ground pelleted diets on broiler performance and DV. Feeding ground pellets allows for thermal processing effects to be demonstrated without being confounded by feed form effects.

MATERIALS AND METHODS

Glucanase Enzyme

Two different experimental, mixer-added gluc enzymes were tested in this experiment: Glucanase A (**GA**) and Glucanase B (**GB**). GA was tested at four doses: 125, 500, 1000, and 2000 Units/kg of feed; and GB was tested at 1000 Units/kg of feed. GA was in an earlier stage of development; therefore, more doses were required to examine enzyme efficacy. GB was a more established candidate; therefore, fewer doses were associated with the enzyme. The enzyme was mixed with a 3 kg sample of complete NC feed in a small paddle mixer then remixed with 500 or 200 kg of the remaining NC complete feed batch depending on the treatment. The remixing took place in a single-screw vertical mixer [15].

Diet Formulations

Diets consisted of positive control (**PC**) and negative control (**NC**) formulation that were based on industry recommendations and digestible amino acid matrices and differed by 150 kcal/kg (Table 1). The NC diet had the gluc enzymes added on top of the formulation at the mixer. A total of 12 diets were fed (Table 2).

Feed Manufacture

All feed was manufactured at the West Virginia University pilot feed mill. A total of 500 kg of feed was batched according to the PC diet formulation. This was then split into two 250 kg

batches. The first 250 kg batch was mixed and fed as PC unprocessed mash. The second 250 kg batch was conditioned using a short-term conditioner (0.31 x 1.30 m, 10-s retention time) [16] with a constant temperature of 80°C and an incoming gauge steam pressure prior to the conditioner of 262 kPa. Pellets were then extruded using a 40-horsepower California pellet mill [17] with a 4.76 (effective thickness) x 38.1 mm (length) pellet die without relief. These pellets were then ground using a roller mill and fed as PC ground pellets. Feeding ground pellets allows for thermal processing effects to be demonstrated without being confounded by feed form effects. A total of 2,400 kg of feed was mixed according to the NC diet formulation. This was then split into six allotments: four allotments contained 500 kg each and two allotments contained 200 kg each. One of the four allotments containing 500 kg was kept as is and the other three were mixed with either GA 125 U/kg of feed, GA 1000 U/kg feed, or GB 1000 U/kg feed. These four allotments were then split into two 250 kg batches, creating a total of eight 250 kg batches. The first batch of 250 kg was mixed and fed as unprocessed mash, and the second 250 kg batch was pelleted following the process described for the PC diet and fed as ground pellets. The two allotments containing 200 kg were either mixed with GA 500 or 2000 U/kg feed, remixed, and fed as unprocessed mash.

Feed Sampling

Average hot pellet temperature (**HPT**), pellet durability index (**PDI**), and percent pellets were recorded as descriptive data during and post manufacture (Table 2). Measures of HPT were obtained by placing an insulated container under the pellet mill, catching hot pellets, immediately closing the lid, and use of a thermocouple thermometer [18] and an 80PK-24 temperature probe. This procedure was performed four times during the run then averaged. Measurements of PDI were obtained using the New Holmen Pellet Tester [19] where one

hundred grams of pelleted samples are subjected to air flow within a perforated chamber for 30 seconds. Measurements of percent pellets were obtained using approximately 2.3 kg of pelleted samples sifted through a Tyler No. 6 screen. The remaining pellets on the screen were recorded as the percentage of pellets for each treatment.

Enzyme Activity

The target enzyme activities for GA were 125, 500, 1000, and 2000 U/kg of feed. The target enzyme activity for GB was 1000 U/kg of feed. A day after manufacture, all 12 dietary treatments were sent to a commercial laboratory for assessment of gluc activity [20]. A dinitrosalicylic acid (**DNS**) assay was performed on the processed and unprocessed feed to determine enzyme activity and retention. This assay uses dinitrosalicylic acid as a color developing agent and measures the amount of β -glucan cleaved by the β -gluc [21]. First, 0.250 ml of substrate solution (Azo-b-glucan, Megazyme, Ireland) was added to 0.250 ml of extract. After 30 min of incubation at 50°C the reaction was stopped by adding 1 ml of precipitant (Na-acetate 3H₂O 4%; Zn-acetate 0.4%; 800 ml methoxyethanol; water to make 1 liter (pH 5)) and vigorous stirring. After 30 min the tubes were centrifuged for 10 min at 1500 x g and the optical density of the supernatant was read at 585 nm. A standard curve was plotted as the optical density released versus the amount of enzyme added (in grams of enzyme per tonne of feed, ppm). The residual enzyme activity of the studied sample was read on the standard curve [21]. Typically this is used on pure enzyme (not in animal feed), but the assay was adapted to use it for feed reliably. The results of the DNS assay can be found in Figure 1.

Live Bird Performance

A total of 960 day-old Cobb x Cobb 500 [22] straight run chicks were obtained from a commercial hatchery [23]. On D1, the chicks were weighed ten birds at a time and were

allocated to 96 raised wire cages based on weight to create uniform initial pen weights. One of the twelve diets was randomly assigned to each pen within a block. A block consisted of 12 adjacent cages, and there were eight blocks or replications. Chicks were housed in raised wire cages in a cross-ventilated, negative-pressure room. Two identical rooms were utilized; each containing 48 cages, creating a total of 96 cages. Room temperature for the day-old chicks was set at 32°C (90°F); and gradually decreased to 29°C (85°F) for the second week and 26°C (80°F) for the third week of the study to create optimal rearing conditions. Feed was placed in external feed troughs and water was supplied through a nipple drinker system; both feed and water were provided for *ad libitum* consumption. Lighting was manipulated through grow-out to ensure that birds had a full gastro intestinal tract when sampled on D21. From D1 to D6 birds were exposed to 24 hours of light, and after D6, the hours of light were decreased gradually until six hours of dark was reached on D20 and 21. On D21, birds were exposed to six hours dark and then allowed to consume feed for four hours to ensure that digesta was present in the digestive tract to perform gut viscosity measurements. The experimental period was a total of 21 days, and measured variables associated with performance included: D1 starting pen weight, D21 ending bird weight, pen FI, feed conversion ratio (**FCR**), bird live weight gain (**LWG**), and pen percent mortality.

Digesta Viscosity Measurements

On D21, three birds from each pen were euthanized via cervical dislocation. The entire digestive tract was removed, and the digesta was squeezed out by hand into a 50 mL centrifuge tube [24]. The digesta was centrifuged [25] at 12,700 RPM for 5 min at 4 °C [26]. A pipette was utilized to transport 1 mL of supernatant into a microcentrifuge tube. The microcentrifuge tube was placed in a 25°C water bath [27] for approximately 10 min. After, 0.5 mL of supernatant

was placed in a Brookfield Cone and Plate Viscometer [28] with a CPE-40 cone and a CPE-44Y cup. Measurements were taken at 30 sec and 1 min at both speeds of 10 and 20 RPM. Similar methodologies were utilized by Lee and coauthors [29].

Statistical Analysis

A Dose X Processing and a Diet Formulation X Processing factorial arrangement of treatments was used to explore main effects and interactions of particular treatments on performance and gut viscosity. The factorial arrangement of treatments may be better appreciated by observing Table 2. In addition, an overall comparison was conducted on all 12 treatments using the Fisher's LSD multiple comparison test. In all analyses a randomized complete block design was utilized with one pen of 10 birds as the experimental unit. All data were analyzed using the GLM procedure of Statistical Analysis System [30]. Alpha was designated as 0.05, and letter superscripts were used to denote differences among treatment means.

RESULTS AND DISCUSSION

Feed Manufacture & Enzyme Activity

A cursory discussion of descriptive data may be useful when considering treatment affects (Table 2). The authors speculate that the PC diet had decreased descriptive HPT (76°C), PDI (55%), and percent pellets (92%) due to the high inclusion of fat that increased lubrication of the pellet die. Variation of formulated fat inclusion was the primary cause for PC and NC difference in energy content. Ground pelleted diets had a decreased descriptive bulk density and increased descriptive average percent moisture compared to the unprocessed mash diets. The particle size differences between the two feed forms were not remarkable.

The descriptive enzyme activity data suggests that GA is more thermally stable compared to GB under the pelleting conditions of the current study (Figure 1).

Live Bird Performance

Glucanase A Dose and Processing. Treatments that included GA were affected by a Dose X Processing interaction for D21 ending bird weight ($P = 0.0465$, Table 3), demonstrating that GA dosed at 1000 Units/kg of feed increased ending bird weight for birds fed ground pelleted diets but not unprocessed mash diets. The authors speculate that the pelleting process changes ingredient confirmation and improved the opportunity for gluc to interact with substrates. In addition, the main effect Processing was significant for D21 ending bird weight, pen FI, and bird LWG ($P < 0.0001$, Table 3 and 4), describing that birds fed ground pelleted diets had a higher ending weight, FI, and LWG compared to birds fed unprocessed mash diets. These results were also observed by Gracia and coauthors [31] where they found that heat processing of barley improved FI and LWG from D 0 to 8 in spite of the increase observed in intestinal viscosity. A Dose X Processing interaction was significant for DV measurements taken at 10 RPM for 30 sec and 1 min ($P < 0.05$, Table 3), demonstrating that GA dosed at 1000 Units/kg of feed decreased DV for birds fed ground pelleted diets. The main effect Processing was significant for DV measurements taken at 20 RPM for 30 sec and 1 min ($P < 0.05$, Table 3), describing that birds fed ground pelleted diets had a higher DV compared to birds fed unprocessed mash diets. Østergård and coauthors [32] discovered that heat processing increased the solubility of the fibrous portion of barley; and therefore, the digesta viscosity was also increased. These scientists also observed that heat processing modifies starch, protein, and fiber structure of the cereal; and consequently, improves accessibility of enzymes to nutrients, facilitating its digestibility [32]. The DV measurements of the current study are approximately

2.0 cP less than DV measurements our laboratory has obtained in high viscous wheat diets that were detrimental to performance [33].

Diet Formulation and Processing. The main effect Diet Formulation was significant for D21 ending bird weight, bird LWG, and FCR ($P < 0.05$, Table 4), describing that birds fed PC had a higher ending bird weight and LWG and a lower FCR compared to birds fed NC, GA 1000 Units/kg of feed, and GB 1000 Units/kg of feed diets. The main effect Diet Formulation was significant for pen FI ($P = 0.0497$, Table 4), demonstrating that birds fed PC and GB 1000 Units/kg of feed had a higher FI compared to birds fed GA 1000 Units/kg of feed. The main effect Diet Formulation was also significant DV measurements taken at 10 and 20 RPM for 30 sec ($P < 0.05$, Table 4), demonstrating that birds fed NC and GA 1000 Units/kg of feed had a higher DV compared to birds fed GB 1000 Units/kg of feed. For DV measurements taken at 10 RPM for 1 min, the main effect Diet Formulation was significant ($P = 0.0069$, Table 4), describing that birds fed GB 1000 Units/kg of feed had a lower DV compared to birds fed NC, PC, and GA 1000 Units/kg of feed. The main effect Diet Formulation was significant for DV measurements taken at 20 RPM for 1 min ($P = 0.0014$, Table 4), describing that birds fed NC and GA 1000 Units/kg of feed had a higher DV compared to birds fed PC and GB 1000 Units/kg of feed.

Overall Comparison. For ground pellets, GA dosed at 1000 U/kg of feed was superior to NC and indistinguishable from PC for ending bird weight and LWG ($P < 0.0001$, Table 5). These benefits were not observed for GB, perhaps in due part to a 50% decrease in enzyme activity post pelleting (Figure 1). Birds fed PC (unprocessed mash and ground pellets) obtained significantly decreased FCR compared to birds fed all other 10 treatments. Birds that consumed ground pelleted diets had greater pen FI compared to birds fed unprocessed mash diets ($P <$

0.0001, Table 5). For DV at 10 RPM at 30 sec and 1 min, NC ground pellet had the statistically highest DV and NC unprocessed mash, GA 125, 500, and 2000 U/kg of feed unprocessed mash, GB 1000 U/kg of feed, GA 1000 U/kg of feed, and PC had the lowest values ($P < 0.05$, Table 5). For DV at 20 RPM at 30 sec and 1 min, NC ground pellet had the statistically highest DV and NC unprocessed mash, GA 125, 500, and 2000 U/kg of feed unprocessed mash, GB 1000 U/kg of feed, and PC had the lowest value ($P < 0.05$, Table 5).

CONCLUSIONS AND APPLICATIONS

1. Descriptive enzyme activity data suggests that GA is more thermally stable compared to GB when conditioned at 80°C.
2. Treatments that included GA were affected by an interaction between Dose X Processing for ending bird weight and DV at 10 RPM ($P < 0.05$). Birds fed GA dosed at 1000 Units/kg of feed decreased DV and increased weight gain for ground pelleted diets; however, these beneficial effects were not apparent for birds fed unprocessed mash diets.
3. The main effect Processing was significant ($P < 0.05$) for pen FI, LWG, and DV at 20 RPM. Birds fed ground pelleted diets had a greater pen FI, LWG, and an increased DV versus mash diets.
4. For ground pellets, GA dosed at 1000 U/kg of feed was superior to NC and indistinguishable from PC for ending bird weight and LWG. These benefits were not observed for GB dosed at 1000 U/kg of feed, perhaps in part due to a 50% decrease in activity post pelleting.

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Table 1. Diet formulations¹ for the negative and positive control diets

Item	Negative control, % inclusion	Positive control, % inclusion
Ingredients		
Barley	45.00	40.00
Soybean meal	26.05	23.87
Corn	17.98	22.77
Soybean oil	4.17	5.01
Porcine meat and bone meal	3.00	5.00
Dicalcium phosphate	1.45	1.26
Limestone	0.87	0.65
Salt	0.40	0.37
DL – methionine	0.32	0.32
Lysine	0.27	0.26
NB3000 ²	0.25	0.25
Threonine	0.11	0.11
Coban 90 ³	0.08	0.08
BMD ⁴	0.05	0.05
Calculated nutrients		
ME (kcal/kg)	2880	3030
Crude Protein (%)	21.29	21.13
Lysine (%)	1.18	1.18
Available Phosphorus (%)	0.45	0.45
Calcium (%)	0.91	0.91

¹Both the positive and negative control diets were formulated based on industry recommendations and digestible amino acid matrices and differed by 150 kcal/kg.

²Vitamin-mineral premix (NB3000, Nutrablend, Neosho, MO) supplied the following per kilogram of diet: manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B₆, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; menadione, 0.83 mg; vitamin B₁₂, 0.01 mg; vitamin E, 16.53 IU; vitamin D₃, 2,133 ICU; vitamin A, 7,716 IU.

³Active drug ingredient monensin sodium, 60 g/lb (90 g/ton inclusion; Elanco Animal Health, Indianapolis, IN) as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria aceryulina*, *Eimeria brunette*, *Eimeria mivati*, and *Eimeria maxima*.

⁴Bacitracin methylene disalicylate, 50 g/lb (50 g/ton inclusion; Alpharma, Fort Lee, NJ), for increased rate of bird weight gain and improved feed efficiency.

Table 2. Feed manufacture variables associated with variation in diet formulation (descriptive data)

Feed Form	Conditioning Temperature (°C)	Trt ¹	Dose ² (Units/kg feed)	Avg Hot Pellet Temp (°C)	Production Rate (tonne/hr)	NHPT ³ 30 sec (%)	Percent Pellets ⁴	Bulk Density ⁵ (kg/m ³)	Avg. Moisture ⁶ (%)	Particle Size ⁷ (microns)	Standard Deviation
Ground Pellet	80	GA	125	77.93	0.834	72.65	96.93	507	14.28	1086	1.80
			1000	77.22	0.790	71.20	97.79	520	13.93	1051	1.82
		GB	1000	76.25	0.786	73.25	95.45	526	13.60	1104	1.78
		NC	---	77.39	0.798	75.10	95.44	521	14.06	1082	1.76
		PC	---	76.11	0.761	55.40	92.32	522	14.03	1094	1.79
Unprocessed Mash	---	GA	125	---	---	---	---	621	12.80	1080	1.93
			500	---	---	---	---	608	12.43	1085	1.96
			1000	---	---	---	---	620	12.52	1022	1.96
			2000	---	---	---	---	617	12.61	1073	1.95
		GB	1000	---	---	---	---	616	12.46	1109	1.95
		NC	---	---	---	---	---	623	12.70	1090	1.93
		PC	---	---	---	---	---	622	12.32	1109	1.99

¹Treatments: PC = positive control; NC = negative control (positive and negative control diets had an energy difference of 150 kcal/kg);

GA = glucanase A; GB = glucanase B

²All enzymes were added to a 2.3 kg of NC diet, mixed, then added back to the larger NC batch, and mixed again.

³New Holmen Pellet Tester: Pellet durability index based on the New Holmen Pellet Tester that uses a sample of 100 g of pellets and air flow within a perforated chamber for 30 s.

⁴Percent pellets were defined as the percentage of crumbles from a 2.3 kg feed sample that did not pass through a No. 6 screen.

⁵Bulk Density data was obtained with a container of known volume.

⁶Average percent moisture data was obtained using AOAC dry matter methodology and duplicate samples.

⁷Particle size was determined with a Ro-Tap particle size analyzer model RX-29 type 110V 60H2, WS Tyler, Mentor, OH. One hundred grams of each ground pelleted diet was placed in a dust-tight enclosed series of stacked (No. 4, 6, . . .) American Society for Testing and Materials (ASTM) screens affixed to the Ro-Tap particle size analyzer and shaken for 10 min. The screens were then separated and weighed. Particle size was calculated by subtracting the weight of the screen from the final weight of screen and sample after shaking. The mean geometric particle size and log normal geometric standard deviation were calculated as described by McElhiney, 1994

Figure 1. Target glucanase activity and DNS assay results

*Dinitrosalicylic acid (DNS) assay: Cosson, T., A. M. Pérez Vendrell, B. González Teresa, D. Reñé, P. Taillade, and J. Brufau. 1999. Enzymatic assays for xylanase and β -glucanase feed enzymes. *Animal and Feed Science and Technology* 77:345-35

Table 3. The effect of Glucanase A dose (0, 125, 1000) and processing (unprocessed mash or ground pellet) on broiler performance and gut viscosity.

Enzyme	Dose (Units/kg feed)	Processing	Day 1 Starting Pen Weight ¹ (kg)	Day 21 Ending Bird Weight (kg)	Pen Feed Intake (kg)	FCR ³ (kg/kg)	Bird LWG ² (kg)	Pen Percent Mortalit y ⁴ (%)	Gut Viscosity (cP)			
									10 RPM (SR ⁵ = 75 sec ⁻¹)		20 RPM (SR ⁵ = 150 sec ⁻¹)	
									30 sec	1 min	30 sec	1 min
Marginal Means												
Glucanase A	0	---	0.469	0.675	9.15	1.47	0.628	0.625	6.28	6.09	5.90	5.73
	125	---	0.462	0.677	9.17	1.46	0.631	0.625	5.57	5.59	5.90	5.79
	1000	---	0.456	0.679	8.98	1.45	0.633	2.50	5.66	5.59	5.82	5.73
	---	Unprocessed Mash	0.467	0.647	8.66 ^b	1.46	0.600 ^b	1.25	5.29	5.23	5.26 ^b	5.14 ^b
	---	Ground Pellet	0.458	0.707	9.84 ^a	1.46	0.661 ^a	1.25	6.38	6.29	6.49 ^a	6.36 ^a
Main Effect and Interaction Probabilities												
Dose			0.4307	0.9240	0.2666	0.6051	0.8626	0.1862	0.4627	0.6093	0.9842	0.9938
Processing			0.2906	< 0.0001	< 0.0001	0.9135	< 0.0001	1.0000	0.0360	0.0293	0.0106	0.0106
Dose X Processing			0.5435	0.0465	0.3787	0.5322	0.0729	0.1862	0.0163	0.0412	0.1525	0.1468

¹Starting pen weights were based on 10 birds per pen.

²Live Weight Gain.

³Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

⁴Mortality percentage is based on 10 birds per pen, so if 2 birds died the mortality percentage would be 20% for that experimental unit/pen.

⁵Shear Rate.

Table 4. The effect of diet formulation (NC, PC, Glucanase A 1000 U/kg feed, Glucanase B 1000 U/kg feed) and processing (unprocessed mash or ground pellet) on broiler performance and gut viscosity.

Diet Formulation	Processing	Day 1 Starting Pen Weight ¹ (kg)	Day 21 Ending Bird Weight (kg)	Pen Feed Intake (kg)	FCR ³ (kg/kg)	Bird LWG ² (kg)	Pen Percent Mortality ⁴ (%)	Gut Viscosity (cP)			
								10 RPM (SR ⁵ = 75 sec ⁻¹)		20 RPM (SR ⁵ = 150 sec ⁻¹)	
								30 sec	1 min	30 sec	1 min
Marginal Means											
Negative Control	---	0.469	0.675 ^b	9.15 ^{ab}	1.47 ^a	0.628 ^b	0.625	6.28 ^a	6.09 ^a	5.90 ^a	5.73 ^a
Glucanase A 1000 U/kg feed	---	0.456	0.679 ^b	8.98 ^b	1.45 ^a	0.633 ^b	2.50	5.66 ^a	5.59 ^a	5.82 ^{ab}	5.73 ^a
Glucanase B 1000 U/kg feed	---	0.464	0.683 ^b	9.29 ^a	1.47 ^a	0.637 ^b	0.625	4.20 ^b	4.10 ^b	4.14 ^c	4.08 ^b
Positive Control	---	0.455	0.708 ^a	9.26 ^a	1.40 ^b	0.663 ^a	0.00	5.35 ^{ab}	5.28 ^a	4.85 ^{bc}	4.78 ^b
---	Unprocessed Mash	0.462	0.660 ^b	8.84 ^b	1.45	0.614 ^b	0.625	5.15	5.03	4.87	4.76
---	Ground Pellet	0.460	0.712 ^a	9.50 ^a	1.45	0.666 ^a	1.25	5.60	5.51	5.49	5.40
Main Effect and Interaction Probabilities											
Diet Formulation		0.3810	0.0048	0.0497	< 0.0001	0.0041	0.0838	0.0075	0.0069	0.0012	0.0014
Processing		0.7192	< 0.0001	< 0.0001	0.7303	< 0.0001	0.3816	0.2776	0.2371	0.0757	0.0609
Diet Formulation X Processing		0.3929	0.0903	0.5927	0.3501	0.1195	0.2847	0.0677	0.1727	0.1565	0.1391

¹Starting pen weights were based on 10 birds per pen.

²Live Weight Gain.

³Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

⁴Mortality percentage is based on 10 birds per pen, so if 2 birds died the mortality percentage would be 20% for that experimental unit/pen.

⁵Shear Rate

Table 5. Overall comparison of all dietary treatments on broiler performance and gut viscosity.

Treatment	Dose (Units/ kg feed)	Processing	Day 1 Starting Pen Weight ¹ (kg)	Day 21 Ending Bird Weight (kg)	Pen Feed Intake (kg)	FCR ³ (kg/kg)	Bird LWG ² (kg)	Pen Percent Mortality ⁴ (%)	Gut Viscosity (cP)			
									10 RPM (SR ⁶ = 75 sec ⁻¹)		20 RPM (SR ⁶ = 150 sec ⁻¹)	
									30 sec	1 min	30 sec	1 min
Negative Control	---	Unprocessed Mash	0.479	0.657 ^c	8.78 ^{cde}	1.46 ^a	0.609 ^c	1.25	5.24 ^{bcd}	5.18 ^{bcd}	4.93 ^{bc}	4.76 ^{bc}
Negative Control	---	Ground Pellet	0.459	0.694 ^b	9.52 ^{ab}	1.47 ^a	0.648 ^b	0.00	7.33 ^a	7.00 ^a	6.88 ^a	6.71 ^a
Glucanase A	125	Unprocessed Mash	0.467	0.645 ^c	8.63 ^e	1.46 ^a	0.598 ^c	1.25	4.46 ^{cd}	4.56 ^{cd}	4.99 ^{bc}	4.90 ^{bc}
Glucanase A	500	Unprocessed Mash	0.457	0.657 ^c	8.80 ^{cde}	1.45 ^a	0.605 ^c	0.00	5.12 ^{bcd}	5.04 ^{bcd}	4.75 ^{bc}	4.65 ^{bc}
Glucanase A	1000	Unprocessed Mash	0.454	0.639 ^c	8.57 ^e	1.46 ^a	0.593 ^c	1.25	6.19 ^{abc}	5.93 ^{abc}	5.85 ^{ab}	5.77 ^{ab}
Glucanase A	2000	Unprocessed Mash	0.457	0.646 ^c	8.72 ^{de}	1.45 ^a	0.600 ^c	0.00	4.29 ^d	4.37 ^{cd}	4.87 ^{bc}	4.81 ^{bc}
Glucanase B	1000	Unprocessed Mash	0.458	0.656 ^c	9.04 ^c	1.48 ^a	0.611 ^c	0.00	3.83 ^d	3.77 ^d	4.11 ^c	4.03 ^c
Glucanase A	125	Ground Pellet	0.457	0.709 ^{ab}	9.71 ^a	1.46 ^a	0.663 ^{ab}	0.00	6.69 ^{ab}	6.61 ^{ab}	6.81 ^a	6.67 ^a
Glucanase A	1000	Ground Pellet	0.457	0.718 ^a	9.38 ^b	1.45 ^a	0.673 ^a	3.75	5.13 ^{bcd}	5.25 ^{bcd}	5.79 ^{ab}	5.70 ^{ab}
Glucanase B	1000	Ground Pellet	0.470	0.709 ^{ab}	9.54 ^{ab}	1.46 ^a	0.662 ^{ab}	1.25	4.57 ^{cd}	4.43 ^{cd}	4.16 ^c	4.13 ^c
Positive Control	---	Unprocessed Mash	0.458	0.690 ^b	8.96 ^{cd}	1.39 ^b	0.644 ^b	0.00	5.33 ^{bcd}	5.23 ^{bcd}	4.58 ^{bc}	4.50 ^{bc}
Positive Control	---	Ground Pellet	0.451	0.726 ^a	9.57 ^{ab}	1.41 ^b	0.681 ^a	0.00	5.37 ^{bcd}	5.34 ^{bcd}	5.12 ^{bc}	5.06 ^{bc}
ANOVA P-value			0.6154	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.1247	0.0067	0.0063	0.0009	0.0006
SEM ⁵			0.009	0.009	0.112	0.013	0.009	0.898	0.626	0.574	0.502	0.478

¹Starting pen weights were based on 10 birds per pen.

²Live Weight Gain.

³Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

⁴Mortality percentage is based on 10 birds per pen, so if 2 birds died the mortality percentage would be 20% for that experimental unit/pen.

⁵Standard Error of the Mean.

⁶Shear Rate.

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Hard working and eager to expand knowledge. Excellent working as an individual, but also works well within a group.

Goal: To acquire animal husbandry and nutrition experience with exotic animals. To one day obtain a career in this field.

EDUCATIONAL RECORD

Master of Science –	2013; West Virginia University
Major:	Nutrition and Food Science
Thesis:	Improving Opportunities for Small Flock Poultry and Efficiency of Commercial Poultry
Bachelor of Science –	2012; West Virginia University
Major:	Animal and Nutritional Sciences

EDUCATION HONORS/AWARDS

Author Publications:

Abstracts

- A. E. Lamp**, K. G. S. Lilly, B. N. Swiger, and J. S. Moritz. 2011 The effect of marine and flaxseed oil inclusion in diets for pastured laying flocks on EPA, DHA, and consumer acceptability of eggs. 2011 Poult. Sci. (Accepted Abstract).
- A. E. Lamp**, K. G. S. Lilly, B. N. Swiger, K. Matak, and J. S. Moritz. 2011 The effect of marine and flaxseed oil inclusion in diets for pastured laying flocks on EPA, DHA, and consumer acceptability of eggs. 18th European Symposium on Poultry Nutrition. (Accepted Abstract & Full Text Submission). Poster will be in Cesme- Izmir, Turkey. November 2011.
- A. E. Lamp**, M. E. Lemons, K. G. S. Wamsley, and J. S. Moritz. 2012 The effect of pasture access, breed, and diet on laying hen health, performance, and EPA and DHA content of eggs. 2012 Poult. Sci. (Accepted Abstract).
- A.E. Lamp**, A.M. Evans, and J.S Moritz. 2013. Feed manufacture technique affects heat transfer to feed that may influence nutritional value. 2013 International Southern Poultry Science Forum. Atlanta, GA. (Accepted Abstract).

A.E. Lamp and J.S Moritz. 2013. The effect of pelleting and glucanase supplementation in barley based diets on feed manufacture, broiler performance, and gut viscosity. 2013 Poult. Sci. (Accepted Abstract).

Co-Author Publications:

Abstracts

M. E. Lemons, K. G. S. Wamsley, **A. E. Lamp**, A. M. Evans, K. J. Shipe, and J. S. Moritz. 2012. Lignosulfonate pellet binder and fat increase broiler feed retention time. 2012 Poult. Sci. (Accepted Abstract).

J.W. Boney, **A.E. Lamp**, and J.S Moritz. 2013. The effects of wheat supplementation to corn and soybean meal based diets on the manufacture of pellets and subsequent turkey performance. 2013 Poult. Sci. (Accepted Abstract).

Graduate Awards and Honors:

- Graduate Student Research Paper Certificate of Achievement, Feed manufacture technique affects heat transfer to feed that may influence nutritional value. 2013 International Southern Poultry Science Forum. Atlanta, GA. (Accepted Abstract).
- Graduate Student Research Paper Certificate of Excellence, The effect of pelleting and glucanase supplementation in barley based diets on feed manufacture, broiler performance, and gut viscosity. 2013 Poultry Science Association Meeting. San Diego, CA (Accepted Abstract).

Attended Marshall University

2008 - 2010

- A. Michael Perry Scholarship (2008-2010)

Graduated from Weir High School in 2008:

- Member of National Honor Society, Weir HS Chapter (2006-2008)
- Salutatorian
- All Conference Academic Award
- Who's Who Among High School Students
- Student Council Treasurer
- USAA National English Award

Scholarships Received:

- Riverside Medical Scholarship (2008-2009)
- Aggarwal Family Scholarship (2008-2009)
- Kristen Andrews Scholarship (2008-2009)
- Stark Foundation Scholarship (2008-2009)
- West Virginia Promise Scholarship (2008-current)

RESEARCH EXPERIENCE

Graduate Research Assistant

Summer 2012 –Present

- Led three contract studies with Verenum
- Led two contract studies with Virginia Poultry Grower's Coop
- Assisted with Contract Studies with Verenum and Lignotech
- Preston County Kid's Safety Day (6/9/2012 and 6/8/2013)
- Assisted with WV poultry week activities (7/26-7/27/2012)
- Poultry Judge for County Fairs (Berkeley County Youth Fair, WV; 8/7/2012) (Monongalia County, WV; 8/9/2012 and 8/1/2013)
- Assisted with Extension talks on backyard poultry production: Monongalia County (8/8/2012) and Gilmer County (10/18/2012)
- Guest Lecturer at Organic Poultry Field Day (8/9/2012)
- Assisted with running of the Poultry Building at the WV State Fair (8/15-8/16/2012)
- Assisted with Marion County Hands-on Ag Day (9/20/2012)
- Assisted with Poultry Career Development Events (9/26/2012 and 6/19/2013)
- Assisted with Family Day at the WVU Animal Science Farm (10/6/2012)
- Presented Poultry Judging lecture for Boy Scout's Animal Science Merit Badge (2/9/2013)
- Presented Feed Manufacture lecture for STEM program (8/7/2013)
- Assisted with Doddridge County Poultry Processing Workshop, WV (August, 2013)

Graduate Teaching Assistant

Fall 2012 –Present

- Teaching Assistant for Poultry Judging Course (ANPR 339)
- Guest Lecturer on Poultry Judging for Principles of Animal Science Class (A&VS 251)
- Teaching Assistant for Companion Animal Science (A&VS 275)

National Meeting Paper Presentations

- 2011 Poultry Science Association (St. Louis, MI) (Undergraduate Student)
"The effect of marine and flaxseed oil inclusion in diets for pastured laying flocks on EPA, DHA, and consumer acceptability of eggs."
- 2012 Poultry Science Association (Athens, GA) (Undergraduate Student)
"The effect of pasture access, breed, and diet on laying hen health, performance, and EPA and DHA content of eggs."
- 2013 Poultry Science Association (San Diego, CA) (Graduate Student)
"The effect of pelleting and glucanase supplementation in barley based diets on feed manufacture, broiler performance, and gut viscosity"

National Meeting Poster Presentations

- 2011 18th European Symposium on Poultry Nutrition (Cesme- Izmir, Turkey) (Undergraduate Student)
“The effect of marine and flaxseed oil inclusion in diets for pastured laying flocks on EPA, DHA, and consumer acceptability of eggs.”
- 2013 International Poultry Scientific Forum (Atlanta, GA) (Graduate Student)
“Feed manufacture technique affects heat transfer to feed that may influence nutritional value.”

Undergraduate Research Assistant

Summer 2011-Summer 2012

- Led three ontract studies with Verenium
- Conducted study “Production of Omega-3 Fatty Acids Enhanced Eggs in a Pastured Poultry System”
- Assisted with Contract Studies with JBS United, Phytex, Verenium, Phytex, Poet Nutrition, Lignotech, and Adisseo
- Assisted with Poultry Workshop at WVU Organic Field Day (8/4/2011)
- Assisted with WV poultry week activities (Summer 2011)
- Assisted with activities and displays (birds and poster) for Monongalia County (Summer 2011)
- Assisted with two contract studies with Poet Nutrition Inc. utilizing various inclusions of dried distillers grains and soluble to establish sparing effects for lysine and available phosphorus (Summer 2011)
- Assisted with Extension talks on backyard poultry production: Doddridge County (2/20/2012), Roane County (2/21/2012), and Wood County (2/23/2012)

Undergraduate Teaching Assistant

Spring 2012

- Teaching Assistant for Poultry Judging Course (ANPR 338)
- Guest Lecturer on Poultry Judging for Principles of Animal Science Class (A&VS 251)

EXPERIENCE

OIT Support Services

2010-2011

- Helped students print large posters
- Helped students with computer problems

Community Care Animal Hospital

2009

- Cleaned cages
- Assisted with animals
- Washed laundry
- Observed surgery
- Restrained animals
- Took x-rays
- Ran fecal floats

Kroger

2009

- Pushed shopping carts from the parking lot into the store
- Bagged groceries for customers

Hancock County Animal Shelter**2008**

- Cleaned cages
- Assisted with animals
- Washed laundry

Heilman Enterprises**2006-2007**

- Completed construction projects

SKILLS

- Internet Literate
- Savvy in Window's Microsoft Programs
- Poultry Handling, Judging and Husbandry
- Feed Manufacture and Diet Formulation
- Precision-feeding
- Cecectomy Surgery
- Tibia Extraction
- Streak-plating for isolated colonies
- Experience with SAS
- Animal/Scientific specific courses taken:
 - Animal Nutrition 260
 - Animal Physiology 301
 - Applied Nutrition 2 362
 - Environmental Microbiology 401
 - Equine Management and Training 281
 - Companion Animal Science 275
 - Comparative Vertebrate Anatomy 310
 - Current Literature in Animal Science 451
 - Intro to Biochemistry 410
 - Poultry Production 367
 - Poultry Judging 338 & 339
 - Principles of Animal Science 251
- Graduate Courses
 - Food Microbiology 545
 - General Biochemistry 610
 - Nutrition and Disease Prevention 614
 - Statistics 511, 512

References available upon request