ABSTRACT

HARDING, KARI LYN. The Effect of Feeding Whole-In-Shell Peanuts, High-Oleic Peanuts, and/or Sweet Potatoes on Laying Hen Performance, Egg Quality and Composition, and Ileal Nutrient Digestibility (Under the direction of Dr. Kenneth E. Anderson).

This thesis evaluated how feeding different alternative ingredients would affect laying hen performance, production, egg chemistry and quality, and ileal digestibility. . For the digestibility study, 64 Shaver hens were randomly assigned to treatments with sixteen birds per treatment. For the effects of peanuts four treatments were examined, a conventional control (C1), and control containing soy protein isolate control (C2), a 4% whole-in-shell peanut (WPS), and an 8% high-oleic peanut (HOPN) diet. After 6 weeks, excreta and ileal samples were collected for analysis. The metabolizable energy corrected for nitrogen (AMEn) was significantly higher in both C1 and HOPN ($P<.0001$) than the other treatments. The apparent nitrogen retention (ANR) was significantly lower ($P=0.0366$) for WPS, but the other treatments had no statistical differences. Apparent fat digestibility was highest ($P<.0001$) for C2 and WPS was lower than HOPN. C1 fed hens had greater apparent protein digestibility than other treatments with HOPN being slightly behind and WPS being the lowest ($P<.0001$). From these results, feeding HOPN to hens proved similar levels to C1 and C2, however when feeding WPS supplementing protein to achieve similar protein digestibility results.

The same diet treatments were fed *ab libitum* to the randomly assigned replicates. Hens were housed 18 per cage with 36 hens per replicate with four replicates per treatment, 576 Shaver hens total. Over six weeks, egg quality and grading was conducted bi-weekly and weeks 0, 2, 4, and 6. Hens fed C2 produced more total dozen eggs compared to C1 over the trial ($P<0.05$). Yolk color scores were significantly higher in eggs produced from C1 and C2 treatments compared to others ($P<.0.05$) but no other egg quality parameters tested were

different. HOPN hens produced eggs with significantly lower stearic and linoleic fatty acid levels than other treatments ($P<0.05$), while eggs from WPS hens had greater β-carotene levels ($P<0.05$). Feeding WPS or HOPN could be a way to enrich the eggs produced without affecting hen performance.

Another digestibility trial examined feeding a conventional control (C1), soy protein isolate control (C2), 4% sweet potato (SWP), 8% high-oleic peanut (HOPN), and 4% sweet potato + 4% high-oleic peanut diet (SWP + HOPN). Treatments were fed to eighty Shaver hens for 6 weeks and excreta and ileal samples were collected for analysis. C1 and HOPN AMEn was superior and SWP + HOPN was lowest ($P<.0001$). The ANR was significantly less ($P=0.0004$) for SWP than other treatments that were not different from each other. SWP+HOPN hens had the highest fat digestibility, and C1 being the lowest with HOPN being just a bit higher ($P<.0001$). The apparent protein digestibility of SWP was higher than all treatments and C1 was lowest ($P<.0001$). Feeding HOPN and/or SWP could be good because of digestibility, but feeding a SWP + HOPN diet may need supplemented with another energy source.

Shaver hens were randomly assigned to one of five diets with 36 birds per treatments and four replicates, 720 hens. Pen body and feed weights were determined bi-weekly and total number of eggs produced per week were calculated for the six week study. Egg quality and grading was conducted bi-weekly and weeks 0, 2, 4, and 6. Hens fed C2 and HOPN produced significantly more eggs ($P<0.01$) than other diets. FCR was better in C1 and C2 than the other treatments ($P<.0001$). Eggs from the SWP diet had higher yolk color at weeks 4 and 6 compared to HOPN and SWP + HOPN ($P<0.01$). The stearic fatty acids were lowest in SWP + HOPN and HOPN treatments ($P<0.01$). Feeding SWP might enhance yolk color, but there are no differences in performance with SWP and/or HOPN.

The Effect of Feeding Whole-In-Shell Peanuts, High-Oleic Peanuts, and/or Sweet Potatoes on Laying Hen Performance, Egg Quality and Composition, and Ileal Digestibility

by
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DEDICATION

To my friends and family that have always had my back and supported me and helped me when needed, and to both my late grandmothers that didn't make it to see me graduate.

BIOGRAPHY

Kari Lyn Harding was born in Charleston, West Virginia on December 15th, 1997 and was raised in Pinch, WV. She is the daughter of Susan and Mark Harding who taught her about hard work, dedication, and kindness. Kari first worked with poultry at a young age when her cousin had backyard poultry, which she helped tend to by feeding, collecting eggs, and hatching the next flock. For most of her life, Kari wanted to become a veterinarian and after graduating high school she attended West Virginia University pursuing an Animal and Nutritional Sciences degree. However, before her junior year, Kari began to work with the WVU poultry lab with manufacturing feed, completing research trials, and lab work. After working with them for about a year, Kari changed her mind and decided to continue on and pursue her master's degree. Kari graduated with her Bachelor of Science in 2020, and accepted a graduate research position under Dr. Kenneth E. Anderson at North Carolina University. She studied the effects of feeding sweet potato, whole-in-shell peanuts and/or high-oleic peanuts on laying hen performance, production, and digestibility. After graduation, Kari plans to pursue her PhD remaining under the direction of Dr. Kenneth E. Anderson.

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CHAPTER 1

Literature Review

Corn and soybean meal are primary ingredients in poultry diets as energy and protein sources, respectively. In the United States, corn has many uses including human consumption, livestock consumption, and ethanol production which will increase its cost and availability (Kreutzer, 2012). Combined with the rapidly growing poultry industry competing for high energy feed ingredients has resulted in an increased cost of feed (MMereale, 2008). Higher feed costs have resulted in a decline in poultry production around the world and caused elevated costs for the consumer. High demands for biofuel and crop shortages around the world have also caused the prices of both feed ingredients and poultry products to increase (Godfray *et al*., 2010; Foresight, 2011; Seppelt *et al*., 2014). These price increases have resulted in a number of countries to shut down their broiler facilities, because they do not have the means to pay for the ingredients they need to grow their poultry. This is tragic due to food security without these facilities (Sakib *et al*. 2014).

Here in the United States, the highest concentration of poultry producers are within the south-eastern states, such as Georgia and North Carolina. However, these states do not grow enough corn or soybeans to provide for the poultry that are produced there. They must import these grains from the Midwest United States, which is expensive, especially with the amount they need to bring in to remain at their high production rate (Economic Research Service, USDA, 2017). According to the most recent statistics North Carolina is nationally ranked number two in turkey production, number four in broiler production, eighth in layer production and has just over 5,700 family owned farms that are producing poultry and eggs. This has an economic impact of approximately $39.76 billion annually (NC Poultry Federation, 2021). To control escalating costs, it is imperative that alternative ingredients are evaluated to determine if they can be fed to our poultry while maintaining performance or product quality.

Whole in Shell Peanuts

According to the USDA statistics, North Carolina ranks number 5 in peanuts produced (USDA, 2020). North Carolina accounts for eight percent of all peanuts that are grown in the southeastern regions in the United States (Archer, 2016). Though in total this is a considerable amount of peanuts produced, unfortunately many peanuts that do not meet the industry's standards for human consumption are crushed for oil or discarded. Of the peanuts' which do not meet food grade, standards include peanut screenings, which includes smaller sized peanuts, and screenings of splits, which account for approximately fifteen percent of production. Peanuts that do not meet that grading or sizing standard requirements that are set forth by the Peanuts Standards Board and the U.S. Department of Marketing Services (American Peanut Council, 2020). While these peanuts do not meet food-grade standards, they are of good nutritional value and quality, and thus might be suitable alternative feed ingredients for the poultry industry. Currently, peanut screenings and/or small grade size peanuts produced are discarded as waste; whereby leading to a possible opportunity for the poultry producers to reduce the feed ingredient importation costs with the utilization of this sector of peanuts as a protein and energy feedstock ration for poultry.

Some peanuts graded for use as feed are further processed by crushing the peanut creating peanut meal, while in developing countries the peanut hull, peanut vines, and peanut skins might also be utilized. Peanut meal is removed from the peanut kernel after oil extraction, creating peanut pulp by-product (Zhao *et al*. 2011). A study shows that using peanut meal can be efficient for poultry performance and egg production (Edwards and Massey, 1941). When compared to other peanut by-products, peanut meal is lower in fiber content and fat. While peanut meal is high in protein content, it needs to be supplemented with threonine, the first limiting amino acid (Pesti *et al*. 2003). Purified threonine is readily available and low cost, so threonine supplementation with peanut meal offsets the cost of corn and soybean and is economical (Pesti *et al*. 2003). Studies have also shown that replacing half of the soybean meal with peanut meal with added fishmeal or lysine in broiler diets, has been shown to have no effect on performance (Driggers and Tarver, 1958).

A laying hen trial comparing birds fed a conventional corn and soybean meal diet and birds fed a peanut meal diet showed similar performance when analyzing feed consumption, egg production, and feed per dozen of eggs (Pesti *et al*. 2003). This twelve-week experiment did find that eggs produced from birds that were fed peanut meal had better interior quality later in their trial compared to the birds fed the conventional diet. The egg Haugh units were analyzed after a two-week storage period and egg from the peanut meal diet had better Haugh unit scores with both refrigeration and room temperature storage (Pesti *et al.* 2003). King and Cottier (1937) demonstrated that feeding peanut meal as the only source of protein to pullets, more eggs were produced, and larger eggs were produced compared to the other peanut by-products that were analyzed. Peanut meal fed birds also produced a firmer fat in the eggs compared to the ground peanuts used due to the higher amount of saturated fat.

It is estimated that for every kg of kernel shell around 35 grams of peanut skins are produced (Zhao *et al*. 2011). The majority of peanut skins are discarded and not utilized. Nevertheless, peanut skins contain polyphenolic and have been used in cattle feed (Sobolev and Cole, 2003). The most common phenolic compounds that are in the peanut skins are procyanidins and catechins which are very active antioxidants (Toomer, 2020a). Peanut skins are made up of 72% carbohydrates approximately 16% of the skins made up of fat, and 12% proteins (Nepote, Grosso, and Guzman, 2002). While they are nutritionally sound, an estimated 0.82 million tons are discarded annually in the United States, with no economic value (Arya, Salve, and Chauhan, 2016).

Previous studies feeding peanut skins to livestock have shown that inclusion rates above 8% lowered the animal's performance because of the procyanidin presence (Hill 2002; Sobolev and Cole, 2003). Procyanidin accelerates the dietary proteins through the tract, which makes them unavailable for ruminants and non-ruminants during digestion (Karchesy and Hemingway, 1988). Feeding peanut skins at over a one percent inclusion rate was shown to result in an added bitter taste to the feed (Hathorn and Sanders, 2012). A study indicated that there was a significant decrease, of seven percent, in crude protein digestibility when there was a fifteen percent inclusion of peanut skins fed to Shiba goats (Saito *et al*. 2016). Abd El-Hack *et al*. (2018) fed a peanut skin powders to broilers and at three weeks of age, chicks fed the peanut skin powder diet had significantly lower feed intake per day however, had no significant impact on feed conversion. They also showed that diets with peanut skins significantly reduced giblet, thigh, and abdominal fat percentages, while increasing breast percentage in 6-week old broilers.

Peanut hulls, or peanut shells as they are more commonly known as, are collected when peanuts that have been graded are put through shelling machines producing peanut kernels and hulls. Peanut hull production is thought to be approximately 230-300 g of peanut hull per kg of peanut (Zhao *et al*. 2011). This is a highly wasted product not only in the United States, but also in China. It's estimated that China is capable of producing a minimum of five million metric tons per year (Wang and Xu, 2008). These hulls can result in a major waste problem and can further pollute the environment where peanuts are still being grown. Currently, peanut hulls cannot be feasibly transported long distances to where they can be utilized because of economic reasons (Zhao *et al*. 2011).

Some previous studies have shown that replacing up to six percent of rice bran with fermented peanut shells would have no effect on poultry performance (Aka *et al*. 2020). Another study has demonstrated that up to five percent of a broiler diet could be replaced by fermented peanut shell meal to increase broiler performance (Armayanti *et al*. 2021). Egbewande (2020) determined that groundnut shells that are treated with alum could replace six percent of wheat offal all while improving carcass yield and maintaining the blood profile of broilers. It has also been thought that by feeding peanut shells, there will be improved gut health because of the insoluble fiber content in the shells. Another trial was run to examine the effects of feeding peanut pods (shells) at different levels and concluded that there was a decreased feed conversion ratio at all of the inclusion rates evaluated. The smallest inclusion of peanut pods at 25 g peanut pod/ kg group and had a higher *Lactobacillus* population compared to the highest inclusion of peanut pods 75 g peanut pod/ kg group. The birds fed the lowest inclusion also had a lower *Escherichia coli (E.coli)* compared to the control group. The highest inclusion showed to have a higher gizzard weight and longer intestinal length and had the lowest pH for the gizzard contents (Navidshad and Aghjegheshlagh, 2018).

It is important to increase the utilization of the waste of these peanut hulls, however a miniscule replacement into conventional diets, may not be financially viable either, because of how much work it is to reduce them to the correct particle size and cost to ship. In some places where production rates for peanuts are high, peanut hulls are already being used as litter, because of their accessibility and low cost. Peanut hull litter has tendencies to cake and be crusty but it is manageable (Ritz, Fairchild, and Lacy, 2009). Some researchers have looked into using peanut hulls as a potential litter source for different animals. One study raised broiler breeder replacement pullets on clean shavings of hull litter and at 20 wks body weight and mortality were not influenced by the litter type, but gizzard weights were decreased on those pullets raised on hulls. Litter moisture in this study were not different, they did however detect aflatoxins in the unused hulls but not the litter. They concluded that these toxins were formed prior to use, however they recommend more research on this topic (Lien *et al*. 1998). In a study comparing different crop residues and how they impacted broiler performance, there were no differences for those raised on groundnut shells showing it can be used as litter (Thirumalesh *et al*. 2012).

Previous research has looked at how different components of the peanut by-products effects poultry production. Many of these studies successfully showed that peanut by-products could replace corn and/or soybean meal either partially, or completely, only one has looked at feeding a whole peanut with its shell to poultry and its effects. This study had two treatments feeding ground peanuts with their shells at an inclusion of fifty percent and twenty-five percent compared to conventional diets as well as ground peanuts without shells, ground peanuts with shells and skim milk, ground peanuts without shells and skim milk, and peanut meal. Birds fed whole peanuts with shell had lower egg production and body weights. When skim milk was an additional protein source, production was higher than both peanut treatments without skim milk. However, it is important to note the vitamin and amino acid analysis for the whole peanuts was unknown (King and Cottier, 1937).

High-Oleic Peanuts

Normal-oleic peanuts have a makeup of 52% oleic acid and a 27% of linoleic acid, which is what has normally been attributed for peanut meal in poultry studies (Pesti *et al*. 2003; Costa *et al*. 2001). Current high oleic peanut cultivars have a greater content of oleic acid at 80% and a

2-3% level of linoleic acid (Braddock, Sims, and O'Keefe, 1995; Toomer *et al*. 2020b). High-oleic peanuts have been found to have a large ratio of oleic acid to linoleic acid, and it was over ten times higher for high oleic peanuts when compared to normal-oleic peanuts. This study used a total of five high oleic lines compared to normal peanuts (Isleib *et al*. 2006). These high levels of oleic acid and lower levels of linoleic acid in high-oleic peanuts positively effects crop yield, with the Georgia-P6G being grown to roughly five thousand seven hundred and forty-six pounds per acre when comparing it with normal oleic-peanuts which are grown at approximately three thousand pounds per acre (AGMRC, 2013; Peanut, 2013).

One of the biggest concerns when feeding peanuts is the potential of aflatoxins reaching levels that could be harmful for livestock. Aflatoxins are two toxic strains growing, *A. parasiticus* Speare, and *Aspergillus flavus* Link ex Fries. In peanut production, aflatoxin contamination can be avoided by following production parameters such as: peak times for harvesting and digging, control of insects that will feast on the shell, and quickly drying what was collected postharvest. Normal oleic-peanuts and high oleic peanuts when analyzed for aflatoxin production and compared showed that all of the high-oleic cultivars that were harvested averaged almost twice the levels of aflatoxin than the normal-oleic peanut cultivars (Xue *et al.* 2003). However, there was an interaction between background genotype and the oleic levels. Overall, the main impact was that background genotype caused a great variance in aflatoxin levels, but does show that special precautions should be taken when feeding high-oleic peanuts to prevent aflatoxin growth (Xue *et al*. 2003).

Previous studies have also investigated the oxidative stability and potential shelf-life of high-oleic peanuts and peanut oil (Braddock *et al*., 1995; O'Keefe *et al*., 1993). It was shown that high oleic peanut oil had better autoxidation when compared to the normal oleic peanut oil.

It also took a significantly longer time period for the high oleic oil to lose half of its triglyceride area on the thin-layer chromatography-flame ionization detector rods compared to the normal oleic peanuts (Xue *et al*. 2003). When analyzing peroxide values in differing storage temperatures, high oleic peanuts had lower values for both temperatures compared to the normal peanuts. The peanut flavor was found to be less stable in the normal oleic-peanuts than high-oleic after being stored for six weeks and determined by a panelist of taste testers. By using the sensory data, shell life was determined to be twice the length for high-oleic peanuts than oleic peanuts, which is important especially for feed production during summer months (Braddock, Sims, and O'Keefe, 1995).

It is important to find a purpose for high-oleic peanuts which will thereby reducing wastefulness. In order to accomplish this, how high-oleic peanuts affects animals and animal products needs to be analyzed. Meyer et al. (1992) found that when feeding high oleic peanuts to pigs, the concentration of lipid oleic acid in the muscle did not affect flavor based on the taste panel scores. A taste panel was set up for a study of chicken breast meat from broilers fed high-oleic peanuts, a conventional control diet, and a control diet with about six percent of oleic acid oil. The chicken breasts were prepared for a blind tasting panel to evaluate the smell, taste, and texture for each of the treatments. Results indicated that the meat was relatively similar for all treatments in all categories with no statistical differences between them (Toomer *et al*. 2019).

This same study conducted by Toomer *et al*. (2019) also analyzed the meat quality of the broilers that were fed high oleic peanuts. The breast yield and the carcass weights of those birds fed high oleic peanuts were lower compared to those fed the conventional diet or the conventional diet plus oleic acid. While there was a reduction in breast yield, there was an increase in leg carcass yields. Overall, it was concluded that when feeding high oleic peanuts, meat pH was reduced with an increase in cooked loss as well as a decrease in light colored values when compared to the other treatments in that trial (Toomer *et al*. 2019).

When feeding high-oleic peanuts to meat birds, it is entirely possible that the amino acid and protein from the meat are influenced differently than when fed a conventional corn and soybean meal diet. High-oleic peanuts could also affect the fatty acid profile of the breast as well as the fat composition that is produce. A recent study found that feeding a conventional diet or a conventional diet with supplemented oleic acid resulted in elevated levels of saturated fatty acids like stearic and palmitic acids and higher trans-fat acid, n9 trans-elaidic acid in the chicken breast when compared to the high oleic peanut fed diet. It was also shown that the high oleic peanut diet in this study contained the lowest amount of cholesterol compared to the other diets and had the greater levels of monosaturated oleic acid (Toomer *et al*. 2020b).

Performance of the broilers fed high oleic peanut showed similar results in body weight as the control diet, but the feed conversion was higher (for this study the lower feed conversion the better) for the high oleic peanut treatments. Feeding high oleic peanuts also was shown to reduce both carcass weights and pectoralis major weights (Toomer *et al*. 2020b). Another study analyzing broiler performance when fed high oleic peanuts resulted in these broilers having significantly lower body weights compared to their other treatments. They also showed similar results as Toomer *et al*. 2020b with the high oleic fed broilers having a higher feed conversion (Toomer *et al*. 2020c).

While some studies have focused more on broiler performance and how this will affect meat quality and taste for consumer consumption, others have focused on how feeding high oleic peanuts as an alternative feed ingredient will affect laying hen performance and their egg quality. It was reported that no significant changes were identified between the high oleic peanut fed hens and the hens fed a conventional diet when analyzing body weights and feed conversion. Egg quality parameters measured were things like number of eggs produced per treatment, Haugh unit, and albumen height, which were found to be not significantly different. However, there were darker yolk color scores for the eggs produced by high oleic peanut fed hens compared to the corn-soybean meal ones (Toomer *et al*. 2019). This is an advantage to feeding laying hens high oleic peanuts, because consumers correlate darker yolk colors with being more nutrient rich compared to lighter yolks. Eggs that came from the high oleic peanut with the skin intact treatment birds had significantly heavier weights than eggs from the corn and soybean meal treatment (Toomer *et al*. 2019).

The composition of eggs produced are also important to look at when feeding a new ingredient. When β-carotene levels were analyzed, the levels in the high oleic peanut treatment were found to be significantly higher, and this could be because of the composition of the fatty acids in the high oleic peanuts. The increase in β-carotene levels relates to the darker yolk color scores to the hens fed high oleic peanuts (Toomer *et al*. 2019). β-carotene is a carotenoid that has been researched and is believed to have great health benefits such as prevention of chronic diseases in humans like type 2 diabetes and reduce the risk of certain cancers, including breast cancer (Woodside *et al*. 2015; Zaheer, 2017). Some studies have tried to supplement diets with carotenoids which in turn would result in the eggs having higher levels of carotenoids like β-carotene, but this method is expensive (Karadas *et al*. 2006; Jiang *et al*. 1994).

Total cholesterol in eggs produced from hens fed high oleic peanuts was significantly greater than the conventional treatment at weeks five and ten during the trial run by Toomer *et al.* (2019). As seen in previous studies, the saturated fatty acids, palmitic and stearic acids were significantly higher in the conventional diet fed birds than the high oleic peanut fed ones at weeks one, five, and ten. The trans-fat n9 elaidic acid was higher in the eggs produced from birds fed the conventional diet at week five and week ten. These eggs also had a significantly higher presence of monounsaturated fatty acid palmitoleic acid and polyunsaturated fatty acid linoleic acid at weeks one, five, and ten (Toomer *et al.* 2019). This is important because consumers demand superior quality and nutrition, therefore research has been directed toward modifying the fatty acid composition, lipid levels and nutrient profile to appease the health minded consumer.

Even though feeding high oleic peanuts would reduce waste generated during processing, the digestibility of the product is important when determining their value as a feed ingredient for poultry. When apparent metabolizable energy was examined in broilers fed a high oleic peanut diet, and a corn and soybean meal diet, the high oleic peanut diet had a higher metabolizable energy profile. This indicates that there was improved uptake of nutrients like dietary fats and carbohydrates. When looking at the histology of the jejunum, there were no differences in villi area for any of the treatments fed (Toomer *et al*. 2020c). The jejunum histology of laying hens when fed high oleic peanuts compared to a conventional diet showed no differences in villi surface area. The apparent metabolizable energy for the high oleic peanut feed for hens was significantly higher as well, as was the ileal protein digestibility (Redhead *et al*. 2021).

One major issue that has come up when feeding peanuts to poultry, or other food production animals, is the potential to transfer of the peanuts allergens to the meat and/or eggs produced for human consumption; which could result in an allergic reaction for peanut-sensitized consumers. Even with proper labeling, mistakes can be made which could result in harm to the consumer and penalties to the company that distributed that product. There are roughly 26 million adults in the United States and eight percent of those are children that have food allergens (Gupta *et al*. 2019). When testing the breast muscle of broilers that were fed either a corn and soybean meal diet, a corn and soybean meal diet with added oleic acid, or high oleic peanuts, all potential peanut and soy protein allergens were undetected. Layer hens were also fed a comparable diet to analyze for allergens within peanut and soy proteins in the eggs they produced, and all showed no indication of either protein allergens (Toomer *et al*. 2020d). This indicates that during digestion the allergens are broken down into their base components rendering them not a threat for consumers that are allergic to peanuts in consumer products from an animal that consumed peanuts, because they do not transfer into the meat or eggs.

Sweet Potatoes

In developing countries it has become increasingly expensive to not only raise livestock, but to feed them appropriately to optimize their growth and performance. Many developing countries cannot keep up with the demand for high protein animal products, because they cannot afford the prices for feed ingredients like corn and wheat bran. To combat this, many areas have turned to alternative ingredients that may be comparable to those currently fed, and will not result in a decline in production or growth performance of the animal. In many of these countries, sweet potatoes are easily obtainable, so the sweet potatoes which cannot be used to feed humans have been used as a livestock feed. Phuc *et al*. (2001) stated that sweet potatoes are one of five of the most important food crops when it comes to total production, coming in behind rice, wheat, maize, and cassava (An, 2004). The top three countries that produce sweet potatoes are China, Brazil, and the United States of America (FAO, 2010).

Over one hundred and five hundred million metric tons of sweet potatoes are produced in the world annually, only one of seven crops to do so (FAO 2011). Almost ninety percent of the world's sweet potato production comes from Asia, with China alone accounting for eighty-five percent of that. Africa produces less than that, accounting for only five percent production (FAO 2010). Sweet potatoes are one of the crops of choice for under and developing countries because of how simple the maintenance for them is. They have a short growing period and require little work for them to be able to thrive. Because of this, sweet potatoes are good food security and have been used to prevent potential famine (Scott *et al*. 1993). Sweet potatoes are well adapted to diverse altitudes which can be up to 2000 ft MSL as well as varying temperatures. They are also better competitors with weeds when compared to other tuber and root crops (Sankaran *et al*. 2012).

Much like peanuts, sweet potatoes have different parts and many of them are fed to livestock around the world. Mainly the roots, vines, foliage, and the waste from processing the starch are fed in parts of Asia and South America. They are also fed in different forms, sometimes depending on the animal being fed, or the farmer that is feeding them (FAO 2011). However, it is interesting to note that the sweet potatoes are produced by the farmer or the village in which the farmer lives and very little are produced at the industry level. Places like China, India, and the Philippines will feed sweet potato parts as is to their cattle, or after it has been mixed with the silage properly (CIP 1998).

Whether the sweet potatoes are fed as is, or sliced and mixed with other feed sources that are handy, all processing is completed in the field. A well accepted procedure to feed sweet potato roots to pigs in Taiwan, is to slice them and allow them to sun dry (Van Soest *et al*. 1991). Some farmers in China will slice the roots increasing digestibility or they will mix them with the sweet potato vines (Chien and Lee, 1980). In Argentina, sweet potato roots that are rejected in the grading process are diverted so they can be fed to swine and cattle, but in Haiti, any roots that are still in the field after harvesting will be dug up by the sounder of swine released into the fields and consumed (Gonzalez *et al*. 2003).

Sweet potatoes are high in carbohydrates and are consumed typically as a source of starch for humans (Beckford and Bartlett, 2015). The dry matter of sweet potato roots is made up of approximately 60-70% of starch (Padmaja, 2009). The high content of starch correlates to roughly 10-30% amylose with the level being dependent on the different strains of sweet potato (Afloayan, 2010). Amylose levels are important to keep in mind when feeding livestock, especially monogastrics, because as the amylose levels increase, it has been shown that digestibility decreases (Zhang and Li, 2004). This happens because the amylase enzymes cannot breakdown amylose at high levels, because the amylose packs tightly together (Waramboi *et al*. 2011). A way to reduce these issues is to further process the sweet potatoes by cooking them in some way, to enhance the susceptibility hydrolysis of the starches during digestion in the animal (Dominguez, 1991).

The sweet potato roots are low in crude protein particularly lysine and essential amino acids that contain and sulfur (Dominguez, 1991). However, sweet potato leaf meal has higher crude protein compared to potato root meal, potato peal waste, and cooked potato meal (Beckford and Barlett, 2015; Hassan and Abd-El Galil, 2013; Unigwe *et al*. 2014; Yakubu, 2014 There has been shown to be a lot of variation in nutrient composition for sweet potato meals, which could be a result of many things such as different types of cultivars, type of fertilizer that was used, as well as the soil types that the sweet potatoes were grown (Khan, 2017). For this reason sweet potato meal is often mixed with other sources of proteins, and vitamin and mineral supplements. Sweet potatoes contain high levels of β-carotene and the carotenoids are found in the sweet potato roots and the concentration ranges from 0 to 20,000 ug/ 100g (Woolfe, 1992).

Other studies have examined the use of ensiling sweet potato roots with nutrient supplementation would affect nutritional values of meat. The carcass and meat values were shown to differ little between treatments aside from pH and fat content, however after being ensiled for nine weeks, the roots did have a better amino acids profile compared to fresh sweet potatoes. Both growing and finishing pigs can be fed sweet potato root meal at inclusions of 54 and 58% respectfully, without reducing weight gain (Gonzalez *et al*. 2002). Three months after feeding swine either cooked, fresh sweet potato roots, uncooked root silage with rice bran, or uncooked roots silage with sun-dried chicken manure, weight gain was not significantly different. Feed efficiency, carcass length, fat thickness, and lean meat contents were not significantly different between treatments (Onwueme and Sinha, 1991). Though it has not been researched, there are claims that by feeding sweet potatoes to pigs, the parasitic load is reduced, resulting in an increased body weight gain (Murugan, Paramasivam and Neunchezhiyan, 2012).

It has been demonstrated that specifically replacing corn with sweet potato is possible for both layers (Bekibele, 1984; Agwunobi, 1993) and broilers (Gerpacio *et al*. 1978; Job *et al*., 1979) will not hinder performance or production. The effects of feeding sweet potato meal to broilers to examine carcass quality, was conducted by Agwunobi (1999) by feeding five different inclusion levels. In the starter diets, the inclusion rates of sweet potato meal were 0, 9, 18, 27, and 36 percent to replace corn. There were no significant differences in feed intake, but the chicks that were fed the thirty-six percent inclusion had about half the weight gain as the control birds did. When switching to the finishing diets, there were only four inclusions which were 0, 15, 30, and 45% replacing corn. The birds on the forty-five inclusion had a poorer weight gain than the control birds, and the control birds had significantly heavier carcass cuts and more abdominal fat than 15, 30, and 45% inclusions.

Therefore, the carcass quality for the birds fed at forty-five percent inclusion were significantly better than the control because of the abdominal fat reduction. It was observed that as the inclusion levels increased, there was a runnier excreta being dropped, which was believed to decrease weight gain. It was concluded that the best inclusion levels were twenty-seven and thirty percent without decreasing body weight (Agwunobi, 1999). Sweet potato root meal has also been used at different levels to determine the performance effects and carcass quality of meat birds. When substituting 0, 10, 20, and 30 percent corn inclusions in four different dietary treatments with sweet potato root meal. The birds fed at the twenty percent level had significantly lower final body weight, body weight gain, and average daily gain compared to those fed thirty percent. Feed conversion ratio was significantly better in all levels being fed sweet potatoes compared to the control birds (Beckford and Bartlett 2015).

Substituting corn for sweet potato meal at 25, 50, 75 and 100 percent in starter broiler diets showed that increasing sweet potato inclusion, body weight gain, feed intake, and feed conversion ratio were reduced, however were not affected when birds were fed the same inclusions in the finisher diet. A higher mortality rate was observed in birds fed sweet potato meal at inclusion levels greater than twenty-five percent (Maphosa *et al*. 2003). Ayuk and Essien (2009) also found that body weight gain, feed intake, and average daily gain were decreased as sweet potato meal inclusions increased.

Previous studies have investigated how feeding sweet potatoes would affect laying hens and their performance and production. Two treatments replaced half of the corn inclusion with sweet potato meal, two others replaced corn fully with sweet potato meal, and two other treatments had wheat bran replaced by half with sweet potato tubers, with the other two replacing wheat bran fully with sweet potato tubers. As the inclusion of both sweet potato meal and sweet potato tubers increased, there was a delay in sexual maturity and an increase in egg weight of the first egg. Hen day production, yolk color, as well as Haugh units were higher in the birds that were fed the fifty percent replacement of maize with sweet potato meal than other treatments tested (Ladokun *et al*. 2007). Aina and Fanimo (1997) studied thirty-six-week-old laying hens when fed either a corn containing diet, or either a cassava meal or sweet potato meal diet where they fully replace corn. They found no significant effects on feed intake, egg weight, shell thickness, Haugh unit, or feed/dozen of eggs or feed/kg egg.

While most studies have focused on the performance and production of the poultry fed sweet potato by-products and have shown it is best to use it to replace corn at certain inclusions and not fully, very few have looked at the digestibility of these by-products. However, one that did look at the protein digestibility of a corn treatment and a treatment where cassava meal replaced all the corn and another where sweet potato meal fully replaced the corn, corn had a significantly better energy digestibility than either of the treatments and cassava meal was significantly worse in regard to the energy digestibility (Aina and Fanimo, 1997). An AME bioassay was conducted after a twenty-one-day feeding trial of sweet potato cultivar to broilers and the value was determined to be 15.39 MJ/kg (Glatz 2007). However, other studies have produced varying results compared to what was found by Ravindran *et al*. (1995) which was found to be 14.54 MJ/kg. This could be due to different types of sweet potato cultivars being used, or because broiler typically have a higher AME than cockerels (Lopez and Leeson, 2007).

Research Justification

The aim of this thesis is to examine the effects of feeding whole peanuts in shells, high oleic peanuts, and sweet potato products on laying hen production and performance. As reviewed above, one study to date has examined the effects of feeding ground peanuts with their shells.

Also, none have looked at the digestibility of whole in shell peanuts and there are limited sources examining high oleic peanut and sweet potato digestibility. The research in following chapters will also examine how feeding these possible alternative ingredients will affect the fatty acid makeup of the eggs produced.

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CHAPTER 2

The Effect of Feeding Whole-In-Shell Peanuts and/or High-Oleic Peanuts to Laying Hens on Ileal Nutrient Digestibility.

ABSTRACT

The search for locally grown alternative ingredients has become a priority over the years to recapture nutrient that could be used for animal feed therby removing them from the waste stream and to minimize the cost of grains and importing them. Though some studies have found that high-oleic peanuts could be used as an alternative to corn, few have looked at their digestibility and none have analyzed the digestibility of whole-in-shell peanuts. This study examines feeding an 8% inclusion of high-oleic peanuts (HOPN), or a 4% inclusion of whole-in-shell peanuts (WPS), compared to a conventional control (C1), and an isolcaloric, isonitrogenous, soy protein isolate containing control (C2). Laying hens were fed over 6 weeks with Celite used as an indigestible marker. The apparent metabolizable energy correct for nitrogen showed significant differences (P<.0001) between treatments with C1and HOPN being higher than either C2 or WPS. The whole-in-shell peanut however was significantly less than C2. There were no differences between C1, C2, and HOPN, however, the WPS apparent nitrogen retention percentage was significantly less than all of the other three treatments (P=0.0336). When analyzing the fat digestibility, the C2 had the highest digestible fat, with the WPS treatment being the lowest (P<.0001). HOPN and C1 were intermediate with the HOPN fed hens had significantly (P<.0001) higher fat digestibility than C1. The apparent protein digestibility of hens fed the C1 diet was significantly greater (P<.0001) than either treatment with HOPN being the next highest. The WPS resulted in the lowest digestible protein percentage compared to the other treatments analyzed (P<.0001). To conclude, feeding high-oleic peanuts could be beneficial because they have comparable apparent metabolizable energy, apparent nitrogen retention, as

well as both apparent fat and protein digestibility to C1. However, it may not be beneficial to feed whole-in-shell peanuts without added protein because of how significantly lower the results have been in all analysis and if fed it may need supplemented protein and/or energy sources.

INTRODUCTION

As demands for corn and corn by-products continue to rise, so does the cost of poulry feed resulting in increased costs of chicken products for the consumer. These poultry feed ingredients are often cost prohibitive in developing countries and consequently other local feed ingredients such as cassava meal, peanut meal, and fish meal are utilized. Studies have shown that feeding peanut meal prepared from normal-oleic peanuts is a good alternative that does not hinder performance (Costa *et al*. 2001; Pesti *et al*. 2003). Some research has shown that feeding high-oleic peanuts could also be a viable alternative in both laying hens as well as broilers (Toomer *et al*. 2019, 2020a, 2020b; Redhead *et al*. 2021). However, there is very little research on their digestibility in poultry and specifically laying hens.

Toomer *et al*. (2020b) examined how feeding high-oleic peanuts (HOPN) affected the ileal digestibility as well as the apparent metabolizable energy (AMEn) when fed to broilers. They found no significant difference in apparent ileal fat, or protein digestibility when comparing a conventional control corn, and soybean meal diet, a high-oleic diet, and a control diet supplemented with 6% oleic acid. The AMEn for the high-oleic peanut treatment was greater than the other two treatments, allowing the authors to conclude that this diet had more apparent digestible energy. Another trial feeding high-oleic peanuts to laying hens reported a higher AMEn compared to those fed a conventional control corn and soybean meal diet (Redhead *et al*. 2021). The ileal digestibility was also significantly higher for the HOPN group compared to the control (Redhead *et al*. 2021).

Most studies examining the digestibility of peanut by-products like shells and skins have focused on ruminants, namely cows. As lactating Jersey cows were fed different diets with increasing percentages of peanut skins (0, 8, 16, and 24% inclusions), it was determined their crude protein digestibility decreased as the inclusion percentages increased (West *et al*. 1993). When lactating cows were fed peanut cake (pressed seeds during oil extraction) in place of a of soybean meal (0, 110, 220, and 330 g/kg of dry matter), it was determined that total digestible nutrients and the digestibility of crude protein, dry matter, and fat were not affected by the substitution at any level of inclusion (Santos Dias *et al*. 2018). However, no studies to date have examined the digestibility of whole-in-shell peanuts in laying hens which may have a benefit in cage free and free-range systems due to the higher heat increment of the feed.

While previous studies have demonstrated the effective utilization of whole in shell high oleic peanuts as an alternative feed ingredient in layers (Harding *et al.* 2021), no studies to date have examined the apparent nutrient digestibility of whole in shell peanuts in the diets of layers. In this study, the objective is to determine the ileal protein digestibility, the apparent metabolizable energy (AMEn), and the apparent fat digestibility.

MATERIALS AND METHODS

This study was conducted in the bird wing of Prestage Department of Poultry Science at North Carolina State University. All methods and procedures used for animal research in this digestibility trial were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC #19-761-A). Sixty-four Shaver laying hens (36-42 weeks) were randomly assorted into single bird cages. The wire cages were PVC coated with dimensions of 30.5 cm X 45.7 providing 1393.9 cm^2/hen. A one week acclimation period was provided for their surroundings before transferring to treatment feed. Hens were assigned to one of four treatment groups in a random manner with sixteen replicates per treatment. The hens were placed on a lighting program of 14:10 L:D and provided feed and water *ad libitum*.

Concept 5 (level 2, version 10.0) was used to formulate the four experimental diets to be isonitrogenous (19.5% crude protein) and isocaloric (2,928 kcal/kg) with an estimated particle size around 800 and 1000 µm (Table 1). A basal control diet (C1) was manufactured using yellow corn and solvent extracted defatted soybean meal. The other control diet (C2) was made using corn and solvent extracted defatted soybean meal and soy protein isolate (ADM Chicago, Illinois, USA). The whole-in-shell peanut (WPS) diet used 8% whole-in-shell high-oleic peanuts and yellow corn and solvent extracted defatted soybean meal. The last treatment was manufactured using 4% unblanched (skin intact) high-oleic peanuts, yellow corn, and solvent extracted defatted soybean meal. The unblanched high-oleic peanuts and whole-in-shell peanuts were run through a Roller mill to reduce particle size to crumbles before their inclusion in their respective diets. All peanut containing diets were determined to be made from aflatoxin free peanuts. All diets were utilized with North Carolina State University (Raleigh, NC, USA) selenium, vitamin, and mineral premixes that were formulated to adhere or surpass the poultry requirements for these components. All diets were analyzed for both aflatoxin and microbiological contaminants by North Carolina Department of Agriculture and Consumer Services and the Food and Drug Protections Division Laboratory (Raleigh, NC, USA). All the feed ingredients and all feed sampled was proven to be free of any microbiological contaminants. Each analysis of experimental diets for crude fat and crude protein values were done by an AOAC-certified lab, ATC Scientific (Little Rock, AR, USA), using AOAC approved standard methods. All experimental diets contained 2% of CELITE (Diatomaceous Earth, Celite Corp, Lompoc, CA) to be an insoluble ash marker in the diets. This was to evaluate the nutrient digestibility with partial excreta collection (Huang *et al*. 2006).

Ileal Digestibility

Treatment diets were fed to the hens for six weeks and ten birds per treatment were randomly selected for fecal collection using pans placed underneath each cage for three days' for each hen. The crude protein (CP) of the feed and excreta was analyzed by ATC Scientific (Little Rock, AR, USA), using AOAC 990.03 methods. The fecal was collected to determine the AME_n, or apparent metabolizable energy corrected for nitrogen, the Apparent Nitrogen Retention (ANR), as well as the gross energy of the fecal. At the end of the experiment, all sixteen birds per treatment were sampled for ileal contents. The ileal contents were collected after separating the gastrointestinal tract from the Meckel's diverticulum to the ileal-cecal junction. The ileum's contents were then gently expressed into conical tubes. Ileum contents and fecal samples were placed in a drying oven at 70°C for 48 hours, and subsequently ground and sieved through a 1-mm screen and stored at room temperature for further analysis. Gross energy of the fecal samples was determined using an adiabatic oxygen bomb calorimeter (IKA model C5003 connecter to compressed oxygen with NESLAB Refrigerated Recirculator CFT-25). Fecal samples were compacted into a pellet using a pellet press. Compacted fecal pellets were weighed and placed into a metal thimble and IKA brand 50J cotton twist for combustion. Samples were sealed in the combustion container and then combusted. Acid Insoluble Ash (AIA) of feed, ileal contents, and, fecal samples were determined using modified methods of (Vogtmann *et al*. 1975). A 2 g sample was collected then boiled in 25mL of HCl, washed with DI water, and filtered using ash-less filter paper. The filter paper and residue were placed into pre-weighed porcelain crucibles and placed in a muffle furnace at 600°C for 6 hours. Samples were allowed to cool slowly then weighed. Apparent metabolizable energy was calculated using the following equation:

$$AME_n = GE_{feed} - (GE_{fecal} \text{ x } AIA_{feed} / AIA_{fecal}) - (8.22 \text{ x } CP_{feacal}/6.25)$$

With:

AME_n = Apparent Metabolizable Energy, corrected for nitrogen
GE = Gross Energy (Bomb Calorimeter)
AIA = Acid Insoluble Ash Recovery

$$ANR = 100 \text{ x } (1-((AIA_{feed} / AIA_{fecal}) \text{ x } (CP_{fecal}/CP_{feed}))$$

With:

ANR = Apparent nitrogen retention
CP = Crude protein

Digestibility % = 100 * (1-((z ileum % / AIA ileum %) / (z diet % / AIA diet %)))

With:

z = one of the measure elements such as protein, fat, etc.
AIA = Acid Insoluble Ash recovery

Total fat content was determined via soxhlet extraction. Approximately 2 g of dried ileal content samples were weighed out onto filter paper and placed in Whatman Cellulose extraction thimbles (26mm x 60mm) and were then put into the Soxhtec System HT 6 1043 extraction unit (Foss Tecator, Sweden). One-hundred mL metal canisters were weighed, and approximately 50mL of diethyl ether was added. The canisters were sealed in place under the thimbles and were boiled at 60°C for 40 minutes. Afterwards, the samples were rinsed for another 40 minutes with valves open and another 20 minutes with the values closed to allow for ether collection. The ether collected in the metal canisters was weighed and recorded.

Statistical Analysis

Each bird served as one experimental unit and all data was analyzed using JMP SAS statistical software (version 9.0) for significance by one-way analysis of variance (ANOVA) with a significance level of $P< 0.05$. If ANOVA results were found to be significant, a Tukey's multiple comparisons t-test was run to compare the means of the treatments against each other.

RESULTS

Analysis comparing the metabolizable energy of all treatments showed that C1 and high-oleic peanut (HOPN) diets had significantly higher metabolizable energy corrected for Nitrogen (AMEn) (P<0.0001) than either the C2 or whole-in-shell peanuts (WPS) diet as shown in Figure 1. The whole-in-shell peanut diet had the lowest AMEn as compared to all treatment groups. Moreover, the apparent nitrogen retention (ANR) was significantly lower (P=0.0366) for the whole-in-shell peanut (WPS) treatment than the other three treatments analyzed. C1, C2, and the HOPN treatments resulted in no statistical differences in comparisons of their apparent nitrogen retention (Figure 2).

The apparent fat digestibility was highest (P<0.0001) for C2 compared to all other treatments with an apparent digestibility percentage of 82.6% as shown in Figure 3. Whole-in-shell peanuts treatment revealed as significantly lower apparent fat digestibility in comparison to all other treatment groups at 61%. While the apparent fat digestibility of the C1 diet was significantly higher than WPS diet, the apparent fat digestibility of C1 diet was significantly lower than the HOPN diet (P<0.0001). Hens fed C1 diet had higher (P<0.0001) apparent protein digestibility percentages than the other treatment (Figure 4). Interestingly, the HOPN dietary treatment had significantly higher apparent protein digestibility than C2 and WPS diets. The WPS diet had the lowest (P<0.0001) apparent protein digestibility in comparison to the other treatments analyzed.

DISCUSSION

Very few studies have analyzed the digestibility of high-oleic peanuts in laying hens (Toomer *et al*. 2020b; Redhead *et al*. 2021) and currently none have focused on the digestibility of whole-in-shell peanuts. Others have interestingly examined the digestibility of peanut components such as the skins, to other animals such as goats (Saito *et al*. 2016). The apparent metabolizable energies for both C1 and HOPN diets were similar and were higher than the C2 and WPS treatment groups, leading to the conclusion that these diets have more accessible dietary energy for the bird to digest compared to the other two treatments, which parallels previous research conducted by Toomer *et al*. (2020b) and Redhead *et al*. (2021). The apparent nitrogen retention percentages were similar between treatments, with the exception of the WPS treatment, which implies that the laying hens fed whole-in-shell high-oleic peanuts had poorer nitrogen retention when compared to the three other treatments.

Apparent fat digestibility was greater in the HOPN treatment as compared to the C1 diet, implying that the dietary fat in the HOPN was more easily digested and consumed in comparison to the C1 and WPS treatment groups. The HOPN diet may have more available fat to digest compared to the other three treatments analyzed. However, a previous study reported no differences in apparent fat digestibility between a HOPN treatment and conventional control layer diet (Redhead *et al*. 2021) or HOPN and conventional control broiler diets (Toomer *et al*. 2020b). While layer production and performance parameters were not adversely affected by the WPS diet (Harding *et al.* 2021), the AMEn, fat and protein digestibility were compromised as compared to the other treatment groups. From these results, it can be concluded that feeding a HOPN diet could be beneficial for providing highly digestible dietary energy and fat. However, if feeding WPS, it could possibly be optimal to supplement protein because of how low the apparent protein digestibility was. Further research needs to be completed before fully ruling out whole-in-shell peanuts since they may have greater in extensive value in extensive production systems which are cooler due to the high heat increment.

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Table 1. Composition of formulated experimental laying hen diets.

	Treatments[1]			
	C1	HOPN	WPS	C2
Feed Ingredients	%			
Corn (yellow)	51.8	51.8	51.3	54.2
Soybean Meal	32.2	27.8	30.2	28.8
Calcium Carbonate	9.6	8.9	9.5	9.6
Dicalcium Phosphate	1.8	2.6	1.9	1.8
Whole In-Shell PN	0.0	0.0	4.0	0.0
High-Oleic PN	0.0	8.0	0.0	0.0
Sodium Chloride	0.25	0.25	0.25	0.25
L-Lysine	0.0	0.08	0.14	0.02
DL-Methionine	0.18	0.20	0.19	0.17
[2]ADM Soy Protein	0.0	0.0	0.0	1.60
Soybean Oil	3.73	0.0	2.26	3.14
[3]Santoquin®	0.05	0.05	0.05	0.05
Choline Chloride	0.05	0.05	0.05	0.05
[4]Mineral Premix	0.20	0.20	0.20	0.20
[5]Vitamin Premix	0.05	0.05	0.05	0.05
[6]Selenium Premix	0.05	0.05	0.05	0.05
ME (kcal/kg)	2928	2928	2928	2928

[1]Four experimental isocaloric (2,928 kcal/kg) and isonitrogenous (19.5% crude protein) diets were formulated: C1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; C2=diet containing defatted soybean meal, corn, and soy protein isolate. Aflatoxin-free peanuts were used in the preparation of all peanut-containing diets.

[2]Soy Protein Isolate provides per kg of diet: crude protein, 14.4 mg; crude fat, 0.64 mg; crude fiber, 0.16 mg; calcium, 0.02 mg; available phosphorus, 0.04 mg; methionine, 0.224 mg; cysteine, 0.192 mg; lysine, 1.01 mg; and threonine, 0.592 mg.

[3]Santoquin®= Feed antioxidant and preservative to prevent fat oxidation in stored feed (Novus International, St. Charles, MO, USA).

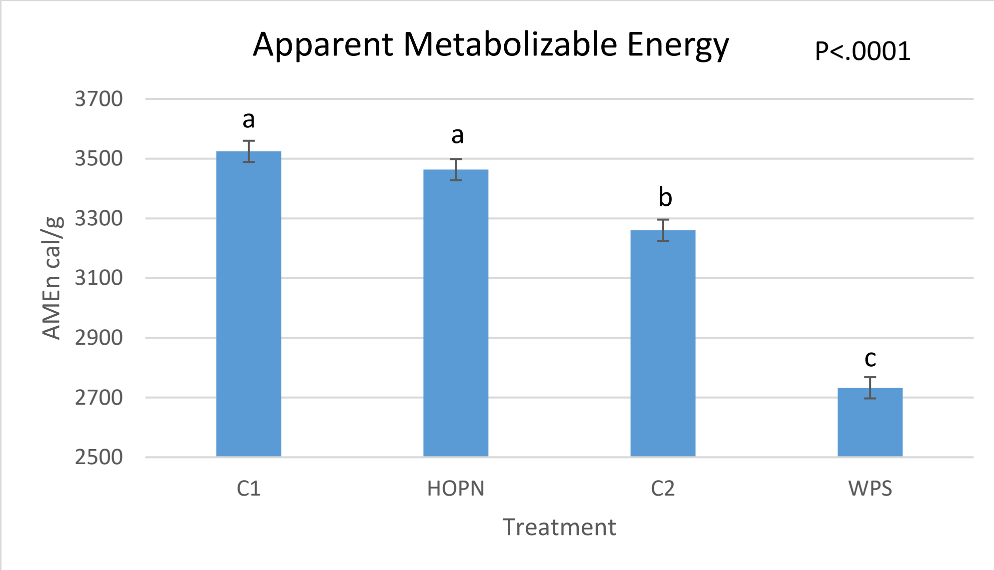
[4]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[5]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B_{12}, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B_6, 55 mg niacin, and 1.1 mg folic acid.

[6]Selenium premix=1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

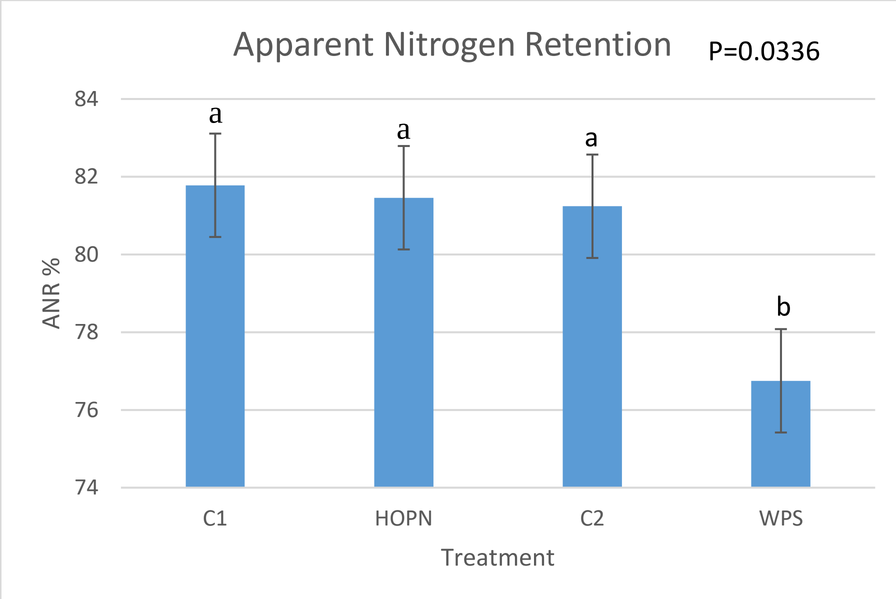
ME=metabolizable energy

Figure 1. The effect of feeding whole-in-shell peanuts and/or high-oleic peanuts to laying hens on apparent metabolizable energy corrected for nitrogen[1].



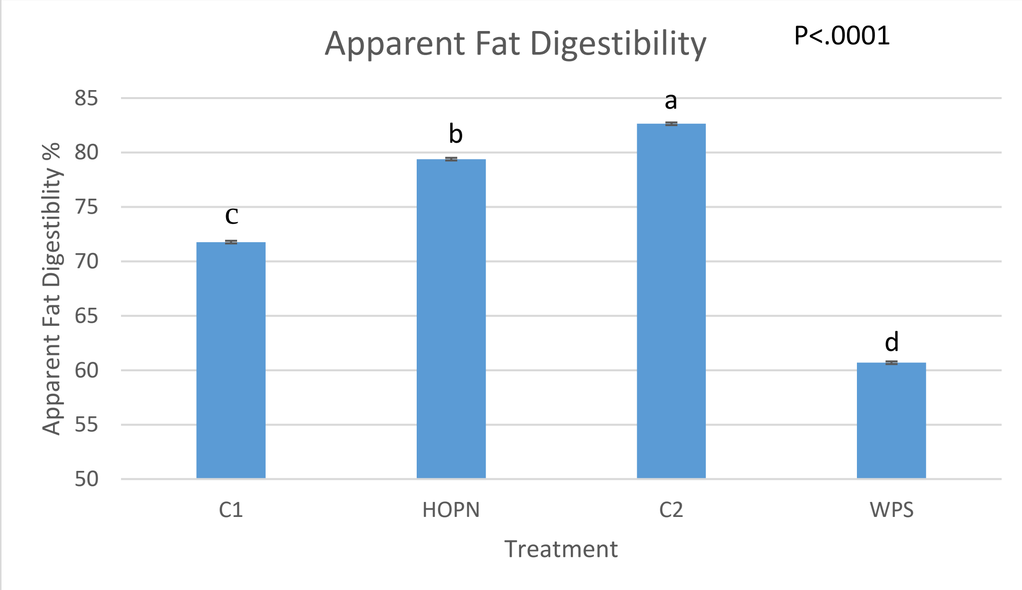
[1]Four isocaloric, isonitrogenous experimental diets containing 2% Celite were fed to 64 laying hens for 6 weeks. Conventional corn-soybean diet (C1), corn-soybean diet including soy-protein isolate (C2), inclusion of 8% unblanched high-oleic peanuts and corn-soybean diet (HOPN), and 4% inclusion of whole-in-shell high-oleic peanuts and corn-soybean diet (WSP). Each bar graphs represents the average ± SE. $AME_n = GE_{feed} - (GE_{fecal} * \text{acid-insoluble ash recovery}_{feed}) - (8.22 * \text{crude protein}_{fecal}/6.25)$. [a,b,c]Bar graphs with different superscripts are significantly different.

Figure 2. The effect of feeding whole-in-shell peanuts and/or high-oleic peanuts to laying hens on apparent nitrogen retention[1].



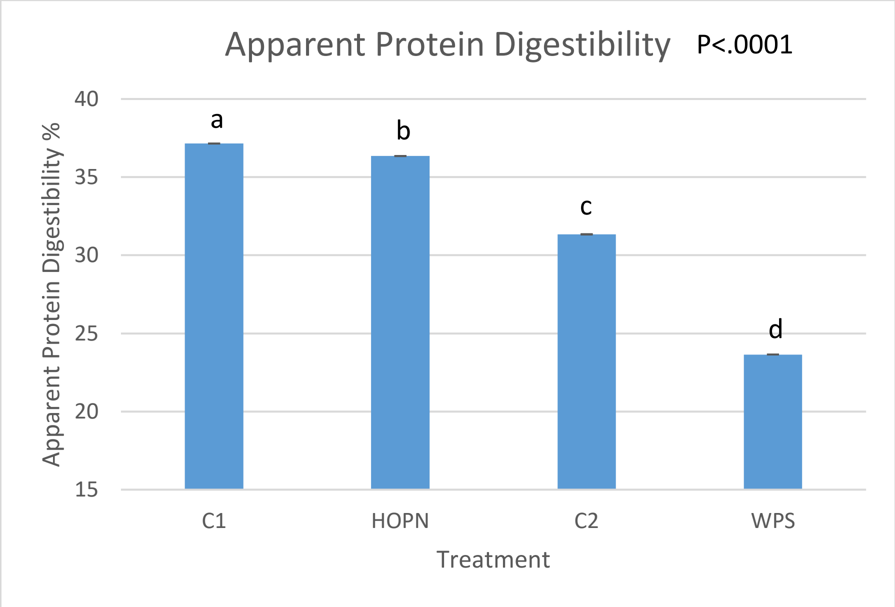
[1]Four isocaloric, isonitrogenous experimental diets containing 2% Celite were fed to 64 laying hens for 6 weeks. Conventional corn-soybean diet (C1), corn-soybean diet including soy-protein isolate (C2), inclusion of 8% unblanched high-oleic peanuts and corn-soybean diet (HOPN), and 4% inclusion of whole-in-shell high-oleic peanuts and corn-soybean diet (WSP). Each bar graphs represents the average ± SE. ANR = 100 * (1-((acid insoluble ash$_{feed}$ / acid insoluble ash$_{fecal}$) * (crude protein$_{fecal}$ / crude protein$_{feed}$)). [a,b]Bar graphs with different superscripts are significantly different.

Figure 3. The effect of feeding whole-in-shell peanuts and/or high-oleic peanuts to laying hens on apparent fat digestibility[1].



[1]Four isocaloric, isonitrogenous experimental diets containing 2% Celite were fed to 64 laying hens for 6 weeks. Conventional corn-soybean diet (C1), corn-soybean diet including soy-protein isolate (C2), inclusion of 8% unblanched high-oleic peanuts and corn-soybean diet (HOPN), and 4% inclusion of whole-in-shell high-oleic peanuts and corn-soybean diet (WSP). Each bar graphs represents the average ± SE. AFD % = 100 * (1-((crude fat_{ileum}/acid insoluble ash_{ileum}) / (crude fat_{feed} / % $Celite_{feed}$))). [a,b,c,d]Bar graphs with different superscripts are significantly different.

Figure 4. The effect of feeding whole-in-shell peanuts and/or high-oleic peanuts to laying hens on apparent protein digestibility[1].



[1]Four isocaloric, isonitrogenous experimental diets containing 2% Celite were fed to 64 laying hens for 6 weeks. Conventional corn-soybean diet (C1), corn-soybean diet including soy-protein isolate (C2), inclusion of 8% unblanched high-oleic peanuts and corn-soybean diet (HOPN), and 4% inclusion of whole-in-shell high-oleic peanuts and corn-soybean diet (WSP). Each bar graphs represents the average ± SE. APD % = 100 * (1-((crude protein$_{ileum}$/acid insoluble ash$_{ileum}$) / (crude protein$_{feed}$ / % Celite$_{feed}$))). [a,b,c,d]Bar graphs with different superscripts are significantly different.

CHAPTER 3

The Effects of Feeding Whole-In-Shell Peanut-Containing Diet on Layer Performance and the Quality and Chemistry of Eggs Produced

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ABSTRACT

The abundance of peanut and poultry production within the state of North Carolina and the US Southeast, led us to conduct a layer feeding trial to determine the utilization of whole-in-shell high-oleic peanuts (WPS) and/or unblanched high-oleic peanuts (HOPN) as an alternative feed ingredient for poultry. To meet this objective, we randomly assigned 576 shaver hens to 4 dietary treatments (4 rep/trt). The dietary treatments consisted of a conventional control diet (C1), diet containing 4% WPS, an 8% HOPN diet, and a control diet containing soy protein isolate (C2). Feed and water were provided for 6 weeks *ad libitum*. Pen body weights (BW) were recorded at week 0 and week 6 (wk6), and feed weights were recorded bi-weekly. Shell eggs were collected daily and enumerated. Bi-weekly 120 eggs/treatment were collected for quality assessment and egg weight (EW), while16 eggs/treatment were collected for chemical analysis. There were no significant differences in BW or EW at wk 6. Hens fed the C2 produced more total dozen eggs relative to C1 hens over the feeding trial (P<0.05). Hens fed the C1 diet consumed less total feed relative to the other treatments with the best feed conversion ratio (P<0.05). Most eggs produced from each treatment were USDA grade A, large eggs. There were no differences in egg quality, with exception to yolk color, with significantly higher yolk color scores in eggs produced from the C1 and C2 treatments relative to the other treatments (P<0.05). Eggs produced from the HOPN treatment had significantly reduced stearic and linoleic fatty acid levels relative to the other treatments (P<0.05). Eggs produced from hens fed the WPS diet had significantly greater

β-carotene content relative to eggs from the other treatment groups (*P*<0.05). In summary, this study suggests that WPS and/or HOPN may be a suitable alternative layer feed ingredient and a dietary means to enrich the eggs produced while not adversely affecting hen performance.

INTRODUCTION

North Carolina is one of the top 6 peanut producing states (Georgia, Florida, Alabama, Texas, North Carolina, South Carolina), which produce nearly all the US annual peanut crop (National Peanut Board, 2021). Relating to this, a considerable quantity of peanuts produced do not meet the industry's standards for human consumption. These components include peanut screenings (small sized peanuts), splits (above the 15%), and any peanuts not meeting the grading or sizing requirements set forth by the Peanut Standards Board and the U.S. Department of Agriculture Marketing Service (American Peanut Council, 2020). Moreover, considerable agricultural waste of peanut shells and skins are produced by the peanut industry during the shelling and blanching process that are discarded due to their little economic value. Peanuts like soybeans are legumes and oilseeds rich in protein, while also providing dietary lipids as energy. Interestingly, peanut skins, the coating of the peanut, provides 15% antioxidant rich polyphenolic compounds, 19% fat and 12% fiber (Toomer et al. 2021). Hence, whole unblanched (skin intact) peanuts may serve as a nutrient-rich value-added alternative feed ingredient for animal food production while reducing the production of agricultural waste by-products from the peanut industry. Thus, numerous studies have been conducted to explore the effective utilization of peanuts and/or peanut by-products within the animal feed as value-added feed components (Hill, 2002; Sobolev and Cole, 2004; Toomer et al. 2019, 2020a, 2020b; Redhead et al. 2021; Toomer et al. 2021a, 2021b) and to enhance agricultural sustainability.

In addition to peanut production, North Carolina is ranked #2 in turkey and #3 in poultry production nationally, with over 5,700 family farms producing poultry & eggs with an economic impact of approximately $39.76 billion annually (NC Poultry Federation, 2021). Importing corn and soybean meal, the primary feed stock rations for poultry, from South America (Crop Prophet, 2019) and even the U.S. Midwest is costly, but if peanuts and/or peanut by-products are shown to be a competitive alternative, a potential reduction in feed costs without hindering growth and performance of poultry could be gained. Using locally grown peanuts (whole in-shell or unblanched) as feed ingredients to the poultry industry could replace imported corn and soybean meal costs. Peanuts could be a viable alternative to soybeans which may have limited availability due to supply and associated rising cost. As a feed ingredient, peanuts and peanut by-products are good sources of protein, fiber, and fat (Toomer, 2018). While a few studies have examined the use of peanut skins (Redhead et al. 2021) and fermented peanut shells (Aka et al. 2020; Armanyanti et al. 2021) as alternative feed ingredients for poultry, no studies to date have examined the use of whole-in-shell peanuts and few studies have examined the use of unblanched peanuts, as a feed ingredient for poultry.

Aka et al., (2020) demonstrated that fermented peanut shells alone could be substituted into feed in place of rice bran at up to 6% inclusion without effecting bird performance, while another study concluded that fermented peanut shell meal could be included up to 5% of the diet to enhance broiler performance (Aka et al. 2020; Armanyanti et al. 2021.) Hence, in this study we aimed to determine the effect of whole-in-shell peanuts and unblanched peanuts on the performance and production of laying hens. Secondly, we aim to determine the effects feeding a whole-in-shell peanut diet or unblanched peanut diet on the quality, lipid and fatty acid content of the eggs produced. In our previous layer feeding trials we demonstrated that layer production performance (body weight, egg weight, egg production, feed intake, and feed conversion ratio) of hens fed a 20% unblanched peanuts diet was similar to the production performance of hens fed a conventional control diet containing soy protein isolate, defatted soybean meal and yellow corn (Toomer et al. 2019). Therefore, in this study we aimed to determine the effects of an 8% unblanched peanut diet on layer production performance and on egg chemistry and quality.

In our previous layer feeding trial, we demonstrated that hens fed a 24% unblanched high-oleic peanut diet had reduced body weights, egg weights, and egg production relative to the hens fed a control diet composed of soy protein isolate, corn, and defatted soybean meal (Toomer et al. 2021). As a consequence, we aim to compare the effects of layer production performance, egg quality and chemistry of hens fed the peanut-containing diets (whole in-shell peanut and unblanched peanut) to a conventional industry standard control diet prepared with defatted soybean meal and yellow corn and a control diet prepared with soy protein isolate, defatted soybean meal and yellow corn to validate these previous findings.

MATERIALS AND METHODS

All animal research procedures used in these feeding trials were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC #19-761-A).

Experimental Design, Animal Husbandry and Dietary Treatments

This study was conducted at the North Carolina Department of Agriculture and Consumer Services Piedmont Research Station (Salisbury, NC, USA). Four experimental diets were formulated in Concept 5 (level 2, version 10.0) to be isocaloric (2,928 kcal/kg metabolizable energy) and isonitrogenous (19.5% crude protein) with an estimated particle size between 800 and 1000 µm (Table 1). Five hundred seventy-six Shaver laying hens (28 to 34 weeks of age) were randomly assigned to one of the four dietary treatments (144 hens per treatment), with four replicates of thirty-six birds per treatment. Hens were housed with 18 birds per cage allowing a space of 175.26 cm^2 per hen. Cages consisted of two rows of a Conventional Tri-Deck Stacked Layer Cage System with 66.04 x 121.92 cm^2 (26 x 48 in^2) per cage. The study was conducted in a standard height, windowless enclosed ventilated house.

Throughout the feeding trial, birds were provided 14 L:10 D and feed and water *ad libitum* for 6-weeks. Pen body and feed weights were recorded bi-weekly. Shell eggs were collected and enumerated daily from each pen and replicate and totaled each week. Total number of eggs produced per replicate for each treatment was calculated for the total 6 week feeding trial. The average feed conversion ratio (FCR) was calculated as total feed consumed over the 6-week feeding trial (kg)/total dozens of eggs produced for each treatment group over the 6-week feeding trial.

The conventional control diet (control-1) was prepared as a conventional layer diet with solvent extracted defatted soybean meal + yellow corn, while the second control diet (control-2) was prepared using soy protein isolate (ADM, Chicago, Illinois, USA) + solvent extracted defatted soybean meal + yellow corn. The whole-in-shell peanut diet (WPS) was prepared using 4% whole-in-shell high-oleic peanuts + solvent extracted defatted soybean meal + yellow corn, and the unblanched high oleic peanut diet (HOPN) was prepared using 8% unblanched (skin intact) high-oleic peanuts + solvent extracted defatted soybean meal + yellow corn. Aflatoxin-free peanuts were used in all peanut-containing experimental diets.

Whole-in-shell high-oleic peanuts were chemically analyzed by ATC Scientific (Little Rock, AR, USA). The nutritional content for whole-in-shell high-oleic peanuts was determined to be: 34.8% crude fat, 22.0% crude protein, 0.16% calcium, 0.30% phosphorous, 34.2% carbohydrates, <5ppm β-carotene, gross energy 6500 kcal/kg using standard AOAC-approved methods for nuts and seeds with crude fat determination using Gravimetric methods for nuts-AOAC 948.22, protein determination using Kjeldahl method for nuts-AOAC 950.48, mineral determination by elemental analysis of mineral by atomic absorption spectroscopy, carbohydrates were determined using standard colorimetric assay determination and spectroscopy, enzymatic-gravimetric methods were used for carbohydrate determination-AOAC 991.43, standard bomb calorimetry methods were used to determine gross energy, and β-carotene was determined using standard high-performance liquid chromatography and spectrophotometry methods (AOAC 948.22, 2018; AOAC 950.48, 2018). Whole-in-shell high-oleic peanuts and unblanched high-oleic peanuts were crushed using a Roller Mill to form crumbles, prior to inclusion in the finished diets. Each of the experimental diets were supplemented with vitamin, mineral, and selenium premixes manufactured at the NC State University Feed Mill (Raleigh, NC, USA) to meet and/or exceed poultry requirements for vitamins, minerals, and selenium. All four experimental diets were fed as mash diets and were analyzed by the North Carolina Department of Agriculture and Consumer Services and the Food and Drug Protection Division Laboratory (Raleigh, NC, USA) for aflatoxin and microbiological contaminants. All feed ingredients and feed samples were verified to be free of microbiological contaminants. All experimental diets were analyzed for crude fat, total cholesterol, fatty acid profile, and β-carotene by an AOAC-certified lab, ATC Scientific (Little Rock, AR, USA), using AOAC approved standard methods described by Toomer et al. (2019).

Egg Quality and Grading

Egg quality was conducted bi-weekly (0, 2, 4, 6) using a 24 sub-sample of eggs randomly selected from each treatment (6 eggs/replicate) in the Egg Quality Lab, Prestage Department Poultry Science, NC State University (Raleigh, NC, USA). Egg quality parameters measured included shell strength, vitelline membrane elasticity (VME), vitelline membrane hardness (VMH), vitelline membrane work of penetration (VMW), egg weight, albumen height, Haugh unit (HU), yolk color, shell color and shell thickness. Eggshell strength was determined using a texture analyzer (TA-HDplus) with a 250 kg load cell measuring in grams of force. The TA-HDplus has a trigger force of 0.02 kg and a testing speed of 1 mm/second. Vitelline membrane strength was determined using the TA.XTplus Texture Analyzer (Stable Micro Systems, Surrey, United Kingdom) with a 1mm blunt probe with a 500-gram load cell per the manufacturer's instructions. The trigger force was 0.0001 kg with a 3.2 mm/second testing speed. Haugh Unit and albumen height were analyzed using the TSS QCD System (Technical Services and Supplies, Dunnington, York, UK). HU is calculated using the following calculation=100Log (h-1.7w + 7.6), with h=egg albumen height and w=weight of egg, with values ranging from 0 to 130 and HU scores below 60 for un-fresh eggs (Nematinia and Abdanan Mehdizaeh, 2018). Yolk color was also determined using the TSS QCD System yolk color scan. Yolk color scan was calibrated using the DSM Yolk Color Fan that determines the color density from lightest to darkest with a range of 1 to 15 (Vuilleumier, 1969). Shell color was determined using refractometry of black, blue, and red wavelengths combined to provide a score from 83.3% (white) to 0% (black). USDA shell egg grading and sizing was conducted on a 120 sub-sample of eggs randomly selected from each treatment group (30 eggs/replicate) bi-weekly.

β-Carotene, lipid, and fatty acid analysis

At week 0 and week 6, a total of 128 eggs (64 eggs at each time point) were randomly selected, with 16 eggs per treatment (4 eggs randomly selected per replicate) for lipid content (total cholesterol, crude fat, and fatty acid profile) and β-carotene analysis by ATC Scientific using AOAC approved methods. Each egg sample was mixed for homogeneity in a Whirl-pak®

(Millipore Sigma, St. Louis, MO, USA) bag for 30 seconds in a Smasher™ Lab Blender (Weber Scientific, Hamilton, NJ, USA), the homogenous egg sample was pipetted into a 50ml conical tube and frozen at -20°C and stored until analysis within 2 weeks of collection. Frozen homogenous egg samples were shipped on dry ice overnight to the vendor for analysis. Total cholesterol, crude fat and fatty acid analysis was conducted using direct methylation methods, as described by Toomer et al (2019). Total cholesterol was measured as mg cholesterol/100 g sample weight (feed or egg), while crude fat was measured as a percentage of gram crude fat/gram sample weight (feed or egg). Fatty acid content was measured as a percentage of gram of fatty acid/gram total lipid content of a sample (feed or egg). Methods used to determine β-carotene content in eggs are detailed in the AOAC 958.05 color of egg yolk method (AOAC 958.05, 1990). Egg fat hydrolysis methods were determined using the AOAC method 954.02 (AOAC 954.02, 1990).

Statistical analysis

Each replicate served as the experimental unit for all variables (body weights, egg weights, feed intake, total dozens of eggs produced, feed conversion ratio). All performance data was evaluated for significance by one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$ using JMP statistical software (version 15.2.1). If ANOVA results were significant ($P < 0.05$), a Tukey's multiple comparisons t-test was conducted to compare the mean of each treatment group with the mean of every other treatment at $P< 0.05$ significance level. The individual egg served as the experimental unit for analysis of all egg quality measurements (120 eggs per treatment, 30 eggs/replicate at each time point) and egg chemistry data (16 eggs per treatment, 4 eggs/replicate at each time point of collection) including crude fat, total cholesterol, fatty acid profile, and β-carotene content between the four treatment groups was conducted for significance by one-way analysis of variance (ANOVA) at a significance level of $P<0.05$ using JMP statistical software (version 9.0).

RESULTS

The formulation of the diets using the analyzed values for the WPS and HOPN ingredients were calculated to be isocaloric and isonitrogenous (Table 1). Chemical analysis of the four dietary treatments confirmed that the formulated diets provided adequate calcium and phosphorous levels for laying hens (Table 2). Dietary palmitic saturated fatty acid, level was the highest in control-1 treatment group. Palmitoleic acid, an omega-7 monounsaturated fatty acid, levels were highest in the HOPN and control-1 experimental diets, relative to the other treatment groups. As expected, oleic monounsaturated fatty acid content was highest in the HOPN and WPS dietary treatments relative to the control group. β-carotene content was negligible (<5ppm) in all experimental treatments and was below the threshold level of assay detection (data not shown). Linoleic acid content was highest in both control dietary treatments relative to the other treatment groups. Linolenic acid content was highest in the control groups and lowest in the HOPN dietary treatment, while homo-gamma-linolenic acid (GLA) levels were similar between all dietary treatments (Table 2). Unexpectantly, elaidic acid content was the highest in the HOPN diet relative to the other treatment groups.

There were no mortalities or health related issues associated with any of the treatment hens during the 6-week feeding trial. There were no significant differences in the variables measured between treatment replicates. Moreover, there were no significant treatment differences in the average pen body weights (Table 3) over the 6-week feeding trial. In this study, there were no significant differences in average egg weights between the treatment groups over the course of this study. However, there were significant treatment differences in hen performance production parameters (Table 4). Hens fed the control-2 diet produced significantly more dozen eggs than the control-1 hens, while hens fed the HOPN and WPS and control-2 produced similar numbers of total dozens of eggs over the 6-week feeding trial ($P<0.05$). Hens fed the control-1 diet consumed significantly less feed relative to the other treatment groups over the 6-week feeding trial Hens of the control-1 and WPS treatment groups had the lowest and most favorable feed conversion ratios, over the course of the 6-week feeding trial.

All eggs produced in this 6-week layer feeding trial had thick, firm egg whites and defect-free egg yolks. All eggshells were clean and without defects, with a minimal number of blood or meat spots (data not shown). Many of the eggs sub-sampled for quality, sizing and (120 eggs per treatment bi-weekly) USDA grading (USDA, 2017) were USDA grade A, large size eggs (Table 5), with greater than 96% Grade A eggs for each treatment. Eighty-six percent of the total 480 eggs sub-sampled (120 eggs sub-sampled bi-weekly) for the WPS treatment group were large size eggs, while approximately 9% were extra-large eggs. Yet, the other treatment groups had 89-90% of the 480 sub-sampled eggs categorized as large size eggs and 4-6% as extra-large eggs (Table 5).

At the onset of the study (week 0), there were significant treatment differences in vitelline membrane elasticity and shell thickness (Table 6). Egg shell thickness was greatest in treatment WPS ($P<0.0001$), and vitelline membrane elasticity was greatest in the controls ($P<0.05$), relative to the other treatment groups at week 0. By week 2, there were no significant differences in vitelline membrane elasticity between the treatment groups.

Shell thickness was the significantly less in eggs from the HOPN treatment group at week 2, relative to the other treatment groups ($P<0.05$). By week 4 of the feeding trial, there were no significant treatment differences in any of the egg quality parameters measured except for yolk color. Egg yolk color was significantly greater in eggs produced from the control groups (control-1 and control-2) relative to the peanut-containing treatment groups (HOPN and WPS) at week 4 ($P \leq 0.01$) of the feeding trial. Also, at week 6 of the feeding trial, egg yolk color was greater in the control groups (control-1 and control-2) relative to the peanut-containing treatment (HOPN and WPS) but was not statistically significant at $P<0.05$.

At week 0 (prior to feeding experimental diets), eggs collected from the HOPN and WPS treatment groups had the highest levels of stearic fatty acid relative to control-1 and control-2 eggs (Table 7). Oleic fatty acid content was significantly reduced in eggs within the control-1 treatment group, relative to the other treatments at week 0. Elaidic acid content was significantly different between eggs of the control-1 and WPS treatment groups, while elaidic acid content was similar between eggs of treatments HOPN, WPS and control-2 treatments at week 0. Linoleic fatty acid content was lowest in eggs of the control-2 treatment group, while linoleic fatty acid content was similar between the other treatment groups at week 0. Nervonic acid content was highest in eggs of the WPS and control-2 treatment groups relative to the control-1 and HOPN treatment groups at week 0. Lastly, omega 3 content was significantly different between eggs of the control-1 and HOPN experimental groups, with the lowest content in eggs of the HOPN treatment group at week 0. Omega 3 levels were similar between eggs of the control-1, WPS and control-2 treatment groups at week 0.

At week 6 of the feeding trial, eggs produced from hens fed the HOPN diet had significantly reduced linoleic and stearic fatty acid content relative to the other treatment groups ($P<0.0001$). This is also parallel the analysis of the experimental diets, with the HOPN diet having the lowest levels of linoleic acid compared to the other treatment groups (Table 2). Nervonic fatty acid content was similar between eggs produced from hens fed the control-1, WPS and control-2 dietary treatments (Table 7), while the nervonic fatty acid content was significantly reduced in eggs produced from hens fed the HOPN diet relative to eggs of the control-1 group (*P*<0.0001). However, nervonic acid levels were very low (0.2 g/kg dry matter) and similar between all experimental diets (data not shown). While there were no statistically significant treatment differences in total omega 3 content between the eggs produced at week 6 of the study (*P*=0.051), eggs produced from hens fed the control-1 diet had highest and eggs produced from hens fed the HOPN diets had the lowest levels of omega 3 content. Interestingly, chemical analysis of the experimental diets (Table 2) demonstrated that total omega 3 content was highest in both control experimental diets and lowest in HOPN (control-1, 63.2 g/kg; control-2, 65.3 g/kg; WPS, 46.1 g/kg; HOPN, 15.1 g/kg dry matter). β-carotene levels were significantly enhanced in eggs produced from hens fed the WPS treatment group, relative to the other treatments at week 6 of the feeding trial (*P*<0.05). In contrast, β-carotene levels were below the assay threshold level of detection (<5ppm) in all experimental diets (data not shown).

DISCUSSION

It was found that the control-1 had the highest level of palmitic saturated fatty acid and HOPN hd the highest levels of palmitoleic acid. *In vivo*, palmitoleic acid is biosynthesized from palmitic acid, and can be found in breast milk, animal fats, and vegetable oils. Palmitoleic acid has been shown to act as an anti-inflammatory, to reduce insulin resistance, and reduce hypertension (de Souza et al 2018). In this study, the homo-gamma- linolenic acid (GLA) levels were not different between treatments. GLA is an omega 6, 18 carbon, polyunsaturated fatty acid found commonly in human breast milk, and in the oils of several botanical seeds and dietary supplementation has been shown to reduce inflammatory biomarkers in several chronic inflammatory disease, such as rheumatoid arthritis and atopic dermatitis (Sergeant et al 2016). There were no differences found in body weights of the hens and this is contrary to our previous layer feeding trials, that showed hens fed the 20% HOPN diet had significantly reduced body weights and egg weights relative to the controls (Toomer et al. 2019), suggesting that higher inclusion levels of HOPN may significantly alter body and egg weights in egg-producing layers. Because there were no significant differences in egg weight, this suggests that inclusion of 8% HOPN or 4% WPS does not alter egg weights relative to conventional eggs.

The WPS treatment group from this study had higher percentages of extra-large eggs compared to other treatments. Most often when purchasing shell eggs from retail stores, egg sizes are determined by the total weight of the dozens of eggs produced into classifications of jumbo to small per the USDA grading shell egg standards (USDA, 2017). Both controls in this experiment resulted in greater vitelline membrane values compared to the other treatments, however there were no significant differences between the treatment groups by week 2. The vitelline membrane is a two-layer transparent protein matrix separating the egg yolk from the albumen and its integrity, strength and elasticity are important quality characteristics required for separation of egg yolk and whites during egg breaking operations (Mertens et al. 2011).

The mechanical properties of the vitelline membrane (strength, and elasticity) are commonly used to determine egg freshness (Mertens et al. 2011). Studies have shown a decline in vitelline membrane strength, and elasticity over time and is dependent upon storage conditions (Jones and Musgrove, 2005; Jones et al. 2002).The egg albumen height is also commonly measured as an indicator of freshness and is measured as the height of the inner thick albumen of the egg when broken on a flat surface and declines with increasing storage time and freshness of the egg (Silversides and Budgell, 2004). The HU (Haugh, 1937) is a measure of egg protein quality based upon the height of the albumen and the weight of the egg as an industry standard to measure egg quality and freshness (Haugh, 1937), with the higher the HU the fresher the egg (Nematinia and Abdanan Mehdizadeh, 2018).

It was determined that egg yolk color was significantly greater in eggs from the control gourps compared to the peanut-containing treatments at weeks 4 and even though not statistically different, they were greater at week 6. This study parallels, our previous reports demonstrating that eggs produced from hens fed a 20% unblanched high-oleic peanut diet had significantly ($P<0.0001$) enhanced egg yolk color in comparison to eggs produced from the controls when analyzed weekly in a 10-week feeding trial, with no other effects seen on egg quality (Toomer et al. 2019). Moreover, in our previous feeding trials there was approximately a 2-fold increase in yolk color of eggs produced from hens fed the unblanched high-oleic peanut diet compared to eggs of the controls ($P<0.0001$) during the last four weeks of the study (Toomer et al. 2019). Feeding trials have demonstrated that egg yolk color is greatly influenced by lipid profile of the hen diet (Suksombat, Samitayotin, and Lounglawan, 2006) and the type and concentration of dietary carotenoids with which are transferred along with their pigments to the yolks of the eggs produced (Leeson and Caston, 2004; Karada et al. 2006).

Eggs that were analyzed at week 6 from HOPN fed hens had significantly reduced linoleic and stearic fatty acid content compared to the other treatments, and the diet analysis also showed the lowest amount. Linoleic acid is the major dietary source of omega 6 polyunsaturated fatty acid found in the Western diet and can be commonly found in vegetable oils, and oilseeds (sunflower, safflower, soybean). In past decades, nutritionists and health care professionals recommended a moderate daily intake of linoleic acid (10% of total energy intake) for the targeted prevention of cardiovascular disease (Harvard School of Public Health, 2017). However, currently there is great debate within the scientific communities regarding the health benefits versus the potential adverse health impacts, such as increased production of pro-inflammatory compounds and increased risk for cancer, associated with increased dietary intake of linoleic acid (Jandacek, 2017).

Stearic acid, followed by palmitic acid, is one of the most found saturated fatty acids found within nature. Stearic acid is an 18-carbon chain found in meat, poultry, dairy products, lard, butter, beef tallow, cocoa butter, coconut oil and palm oil (Berry, 2009). In decades past, high intakes of dietary saturated fatty acids increased the risk of cardiovascular disease (Berry, 2009). However today new studies report that moderate intake of stearic acid does not increase the risk for cardiovascular disease and may reduce low density-lipoprotein cholesterol (Senyilmaz-Tiebe et al. 2018). Nervonic fatty acid was significantly less in HOPN eggs than the control group, while the diets had similar levels. Nervonic acid is a very long fatty acid (24 carbon chain) monounsaturated omega 9 fatty acid, with its name derived from its discovery within mammalian nerve tissue. *In vivo*, nervonic acid combines with sphingosines to form sphingolipids which are important components of brain white matter and thus important to neural health (Li et al. 2019). Nervonic acid is found in some the seed oil of some wild plants but is also biosynthesized by elongation of oleic acid within the endoplasmic reticulum membrane (Li et al. 2019).

β-carotene levels were significantly higher in eggs produced from the WPS fed hens compared to other treatments at week 6, while in the diets, levels were below the level of detection. Extracted peanut oil has been reported to contain small quantities of β-carotene (Al Juhaimi et al. 2018). Pattee and Purcell, (1967) demonstrated that peanut oil extracted from young peanuts contained 60 µg of β-carotene and 138 µg of lutein per liter, while peanut oil extracted from more mature peanuts had lower concentrations (Pettee and Purcell, 1967).

Peanuts have an indeterminate growth pattern, and thus at harvest peanut pods are collected from a range of differing maturity levels on the peanut plant. Harvest containing a higher percentage of young pods may have elevated levels of β-carotene and lutein in the seed of peanuts. Hence, the whole-in-shell peanuts (WPS) utilized in this study may potentially have had a higher percentage of young peanuts containing elevated levels of β-carotene which enriched the eggs produced in this treatment group. In contrast, our previous feeding trials demonstrated that eggs produced from hens fed a HOPN diet had enhanced β-carotene content and yolk color relative to the conventional control eggs (Toomer et al. 2019). Also, in our previous, eggs produced from hens fed a 20% unblanched high-oleic peanut diet had approximately a 1.5-fold increase in β-carotene content relative to the conventional control eggs at week 5 and week 10 of the feeding trial ($P<0.0001$).

Most importantly, this study reports similar body weights, feed intake, FCR and egg weights between the peanut containing diets (4% WPS and 8% HOPN diets) to both control diets implying the effective utilization of whole-in-shell high-oleic peanuts and/or unblanched high-oleic peanuts as alternative layer feed ingredients at these inclusion rates. This study supports the use of peanuts as a value-added feed ingredient to support poultry and egg production within North Carolina and the US Southeast, and agricultural sustainability. Moreover, we demonstrated that there were no treatment differences in any of the variables measured between the control-1 and control-2 treatment groups, which advocates for the use either as a suitable dietary control in future layer feeding trials. While this study has positive implications for the use of peanuts as an alternative layer feed ingredient, this feeding trial must be replicated to more closely parallel commercial egg production methods.

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Table 1. Composition of formulated experimental laying hen diets.

	Treatments[1]			
	Control-1	HOPN	WPS	Control-2
Feed Ingredients	g/kg DM			
Corn (yellow)	518.4	518.0	512.5	542.0
Soybean Meal	322.4	277.9	301.6	288.3
Calcium Carbonate	95.5	88.9	95.0	95.8
Dicalcium Phosphate	18.1	26.0	18.6	18.1
Whole In-Shell Peanut	0.0	0.0	40.0	0.0
High-Oleic Peanut	0.0	80.0	0.0	0.0
Sodium Chloride	2.5	2.5	2.5	2.5
L-Lysine	0.0	0.8	1.4	0.16
DL-Methionine	1.8	2.0	1.9	1.7
[2]ADM Soy Protein	0.0	0.0	0.0	16.0
Soybean Oil	37.3	0.0	22.6	31.4
[3]Santoquin®	0.5	0.5	0.5	0.5
Choline Chloride	0.5	0.5	0.5	0.5
[4]Mineral Premix	2.0	2.0	2.0	2.0
[5]Vitamin Premix	0.5	0.5	0.5	0.5
[6]Selenium Premix	0.5	0.5	0.5	0.5
ME (kcal/kg)	2928	2928	2928	2928

[1]Four experimental isocaloric (2,928 kcal/kg) and isonitrogenous (19.5% crude protein) diets were formulated: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. Aflatoxin-free peanuts were used in the preparation of all peanut-containing diets.
DM=dry matter.
[2]Soy Protein Isolate=purchased from ADM, Chicago, Illinois, USA
[3]Santoquin®= Feed antioxidant and preservative to prevent fat oxidation in stored feed (Novus International, St. Charles, MO, USA).
[4]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.
[5]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B_{12}, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B_6, 55 mg niacin, and 1.1 mg folic acid.
[6]Selenium premix=1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.
ME=metabolizable energy

Table 2. Chemical and fatty acid analysis of experimental laying hen control and peanut-containing diets.

	Treatments[1]			
	Control-1	HOPN	WPS	Control-2
Nutrient	g/kg DM			
Crude fat[2]	56.4	80.7	65.7	52.2
Calcium	28.2	33.4	35.0	33.8
Phosphorus	6.30	7.60	6.80	6.70
Palmitic (16:0)*	123	95.0	95.6	109
Palmitoleic (16:1)*	7.50	6.90	1.60	3.50
Stearic (18:0)*	43.4	30.7	34.90	38.50
Oleic (18:1)*	230	593	394.0	219.0
Elaidic (C18:1 trans)*	0.50	0.80	0.40	0.90
Linoleic (18:2)*	510	198	390	533
Linolenic (18:3)*	62.1	12.8	44.6	63.4
Total Omega 3	63.2	15.1	46.1	65.3
Homo-ɣ-linolenic (18:3n-6)*	0.40	0.70	0.60	0.60

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. Four dietary treatments were chemically analyzed by AOAC-certified lab, (ATC Scientific, Little Rock, AR, USA) using standard AOAC-approved methods. [2]Crude Fat content = g crude fat/g total sample weight * 100, *Fatty acid content = g of fatty acid/g total lipid content * 100.

Table 3. Body weights of hens fed a control or peanut-containing diet.

	Treatments[1]					
	Control-1	HOPN	WPS	Control-2	SEM	*p*-value*
	(kg)					
Week 0	1.64	1.64	1.67	1.66	0.02	0.07
Week 2	1.57	1.58	1.60	1.57	0.02	0.42
Week 4	1.58	1.66	1.65	1.62	0.04	0.32
Week 6	1.61	1.64	1.66	1.64	0.03	0.46

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate.
576 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 4 treatments with 4 replicates/treatment and provided feed and water *ad libitum* for 6-weeks. Body weights were recorded bi-weekly for each pen (18 hens per pen). Each value represents the mean pen weight ± the standard error. *p-value=statistically significant differences $p<0.05$ by analysis of variance (ANOVA).

Table 4. Production performance per replicate of hens fed a control or peanut-containing diet[1].

Treatments	Total Dozen Eggs Produced	Total Amt Feed Consumed (kg)	Total Amt Feed Consumed/bird (kg/hen)	FCR (kg total feed consumed/total dozen eggs produced)[2]	Egg Wt.(g)[3]
Control-1	120[b]	152[b]	4.23[b]	1.26[b]	57.1
HOPN	126[ab]	169[a]	4.70[a]	1.34[a]	57.1
WPS	122[ab]	163[a]	4.52[a]	1.26[b]	57.3
Control-2	128[a]	161[a]	4.48[a]	1.33[a]	57.9
SEM	1.40	2.10	0.06	0.009	0.29
P-value*	0.01	0.0008	0.0008	<0.0001	0.2061

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate.
576 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 4 treatments with 4 replicates/treatment and provided feed and water *ad libitum* for 6-weeks. Eggs were collected daily and enumerated weekly from each replicate pen (4 reps/treatment). Pen body weights were collected with ≈18 hens/pen. Feed intake was calculated weekly for each pen (≈18 hens/pen).
[2]FCR= feed conversion ratio calculated as kg total feed consumed over the 6-week/total dozen eggs produced over 6-week feeding trial per replicate for each treatment (4 reps/treatment). Each value represents the replicate mean ± the standard error. **P*-value=statistically significant differences $P<0.05$ by analysis of variance (ANOVA).
[3]Egg Wt=egg weights represent average egg weights per replicate for each treatment (4 reps/treatment).

Table 5. Size and USDA grading of shell eggs produced per replicate of hens fed a control or peanut-containing diet[1].

	Treatments[1]					
	Control-1	HOPN	WPS	Control-2		
		(%)*			SEM	P-value
Grade A	96.50	96.30	97.90	98.10	1.58	0.77
Grade B	1.88	3.13	0.00	0.00	1.58	0.45
Cracks	1.67	0.63	2.08	1.88	0.62	0.39
Mechanical Loss	0.00	0.00	0.21	0.00	0.10	0.43
Extra-Large	5.83	4.17	8.96	5.00	2.07	0.42
Large	89.00	90.00	86.04	90.83	1.24	0.08
Medium	4.38	5.42	3.96	3.96	1.52	0.89
Small	0.83	0.42	0.83	0.21	0.53	0.80

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate.
576 white Shaver laying hens (28 to 36 weeks of age) were assigned to one of 4 treatments (4 replicates/treatment. Bi-weekly (week 0, 2, 4, 6), a sub-sample of 120 eggs (30 eggs/rep) per treatment were assessed for USDA grading and sizing for a total 480 eggs. *Percentage of the 480 egg sub-sample per treatment.

Table 6. Quality of eggs produced per replicate of hens fed a control or peanut-containing diet.

		Treatment[1]					
		Control-1	HOPN	WPS	Control-2	SEM	*P*-value*
Wk0							
	Shell Sth. (g force)	5298	5011	5346	5442	238	0.308
	SD (mm)	0.29[a]	0.27[ab]	0.25[b]	0.26 [ab]	0.01	0.001
	VMS (g)	2.18	1.94	2.22	2.12	0.15	0.247
	VME (mm)	1.70	1.41	1.78	1.68	0.18	0.205
	Shell Color (%)	82.3	83.3	83.8	84.7	1.0	0.149
	Albumen Ht. (mm)	8.15	8.49	8.22	8.45	0.29	0.593
	Haugh Unit (HU)	90.9	92.5	91.0	92.3	1.6	0.614
	Yolk Color (1-15)	1.88	2.17	2.21	2.21	0.21	0.335
	Shell Thick (mm)	0.37[c]	0.38[c]	0.45[a]	0.44[b]	0.11	<0.0001
Wk2							
	Shell Sth. (g force)	5473	5350	5440	5432	247	0.97
	SD (mm)	0.235	0.232	0.241	0.241	0.01	0.34
	VMS (g)	2.45	2.40	2.45	2.22	0.18	0.18
	VME (mm)	1.96	1.94	2.0	1.75	0.21	0.64
	Shell Color (%)	83.3	81.6	83.9	83.2	0.86	0.06
	Albumen Ht. (mm)	8,85	8.47	8.44	8.73	0.32	0.46
	Haugh Unit (HU)	94.7	92.6	91.1	94.4	2.2	0.36
	Yolk Color (1-15)	2.83	2.50	2.83	2.88	0.17	0.10
	Shell Thick (mm)	0.39[a]	0.37[b]	0.39[a]	0.39[a]	0.01	0.03
Wk4							
	Shell Sth. (g force)	5466	5582	5428	5554	236	0.90
	SD (mm)	0.217	0.222	0.217	0.226	0.01	0.55
	VMS (g)	2.31	2.48	2.32	2.32	0.15	0.62
	VME (mm)	1.91	2.09	1.92	1.91	0.19	0.69
	Shell Color (%)	84.7	85.0	84.4	85.5	0.75	0.49
	Albumen Ht. (mm)	8.71	8.61	8.56	8.43	0.21	0.60
	Haugh Unit (HU)	93.5	93.0	92.6	92.0	1.10	0.58
	Yolk Color (1-15)	2.96[a]	2.67[b]	2.79[b]	3.08[a]	0.13	0.01
	Shell Thick (mm)	0.40	0.40	0.40	0.39	0.01	0.33
W6							
	Shell Sth. (g force)	5252	5164	5675	5282	251	0.18
	SD (mm)	0.214	0.226	0.226	0.217	0.01	0.56
	VMS (g)	2.29	2.40	2.17	2.20	0.14	0.39
	VME (mm)	1,89	2.02	1.75	1.77	0.18	0.42
	Shell Color (%)	83.7	84.1	83.4	84.3	0.72	0.56
	Albumen Ht. (mm)	8.34	8.26	8.50	8.33	0.24	0.79
	Haugh Unit (HU)	91.5	90.9	91.5	91.5	1.20	0.94
	Yolk Color (1-15)	2.92 ±	2.50	2.54	2.79	0.17	0.046
	Shell Thick (mm)	0.39	0.38	0.40	0.39	0.01	0.22

576 white shaver laying hens (28 to 34 weeks of age) were assigned to one of 4 treatments with 4 replicates/treatment and provided feed and water *ad libitum* for 6-weeks. 24 eggs per treatment (6 eggs/replicate) were randomly selected and analyzed at each time point for quality assessment.

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. Bi-weekly at 120 sub-sample of eggs were collected from each treatment group for quality assessment using Technical Services and Supplies QCD system, with calibration with the DSM Color Fan for yolk color. Yolk color=index 1-15 (lightest to darkest color intensity). Shell Sth=shell strength; SD=shell deformation; VMS=vitelline membrane strength; VME=vitelline membrane elasticity; Albumen Ht.=albumen height; Shell Thick=shell thickness. Each value represents the bi-weekly average ± the standard error with 120 eggs/treatment. **P*-value=statistically significant differences $P<0.05$ by analysis of variance (ANOVA). [a,b,c]Means within the same column lacking a common superscript differ significantly ($P<0.05$).

Table 7. The β-carotene, lipid and fatty acid analysis of eggs produced from hens fed a control or peanut-containing diet.

	Treatments[1]					
	Control	HOPN	WPS	Control-2	SEM	*P*-value*
Wk0						
Crude Fat	5.23	4.33	5.28	6.36	0.70	0.061
Palmitic (16:0)	22.9	23.2	23.3	22.2	0.36	0.28
Stearic (18:0)	9.24[b]	9.58[ab]	9.95[a]	9.33[b]	019	0.007
Oleic (18:1)	28.4[b]	29.6[a]	29.8[a]	29.8[a]	0.48	0.030
Elaidic (C18:1 trans)	0.18[a]	0.09[ab]	0.06[b]	0.10[ab]	0.03	0.032
Linoleic (18:2)	24.3	24.5	25.3	23.9	0.49	0.08
Nervonic (24:1, n−9)	1.03[b]	1.06[b]	1.12[a]	1.16[a]	0.03	0.002
Omega 3 (18:3)	1.84[a]	1.55[b]	1.58[ab]	1.71[ab]	0.10	0.03
Cholesterol	192	175	221	253	40	0.23
β-carotene	2.02	1.99	2.19	2.03	0.22	0.69
Wk6						
Crude Fat	6.09	7.08	7.31	5.80	0.94	0.32
Palmitic (16:0)	21.4	21.2	21.3	21.8	0.50	0.64
Stearic (18:0)	8.63[a]	7.03[b]	8.13[a]	8.36[a]	0.30	<0.0001
Oleic (18:1)	30.3	37.6	35.4	37.2	5.80	0.56
Elaidic (C18:1 trans)	0.16	6.84	0.14	0.11	5.30	0.46
Linoleic (18:2)	20.4[a]	11.8[b]	18.2[a]	17.4[a]	1.50	<0.0001
Nervonic (24:1, n−9)	1.08[a]	0.73[b]	0.97[a]	0.88[ab]	0.51	<0.0001
Omega 3 (18:3)	1.76	1.04	1.35	1.19	0.26	0.051
Cholesterol	264	244	326	252	39	0.189
β-carotene	2.41[b]	2.49[b]	3.82 [a]	2.17[b]	0.57	0.043

576 white shaver laying hens (28 to 34 weeks of age) were assigned to one of 4 treatments with 4 replicates/treatment and provided feed and water ad libitum for 6-weeks. 16 eggs/treatment were chemically analyzed at each time point of collection.

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; WPS=diet containing defatted soybean meal, corn and 4% whole in shell high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. At week 0 (wk0) and week 6 (wk6) of the feeding trial, 4 eggs were randomly selected per replicate (16/treatment) and chemically analyzed for lipid content (cholesterol, crude fat), fatty acid, and β-carotene content by an AOAC-certified lab (ATC Scientific, Little Rock, AR, USA), using AOAC approved standard methods. Each value represents the mean ± the standard error with 16 eggs/treatment. **P*-value=statistically significant differences $P<0.05$ by analysis of variance (ANOVA). [a,b,c]Means within the same column lacking a common superscript differ significantly ($P<0.05$).

CHAPTER 4

The Effect of Feeding Sweet Potato and/or High-Oleic Peanuts to Laying Hens on Ileal Nutrient Digestibility

ABSTRACT

As the evaluation of alternative ingredients continues, it is important to understand the digestibility those ingredients that have been proven to be good alternatives really are. Very few studies have examined how digestible high-oleic peanuts and sweet potatoes are when fed to laying hens. The goal of this study was to determine between diets the ileal digestibility by analyzing the apparent metabolizable energy corrected for nitrogen, apparent nitrogen retention, apparent fat digestibility, and apparent protein digestibility. Five isocaloric, isonitrogenous diets were fed to eighty Shaver laying hens for six weeks. Each diet had a 2% inclusion of Celite marker. The five diets were a conventional corn-soybean control (control-1), a corn-soybean plus soy protein isolate control (control-2), an 8% inclusion of high oleic peanut treatment (HOPN), a 4% inclusion of sweet potato (SWP), and a 4% sweet potato inclusion + 4% inclusion of high-oleic peanut (SWP + HOPN). The control-1 and high-oleic peanut fed birds had greater metabolizable energy results compared to the other treatments meaning the energy from these diets was more accessible for the birds to digest while the sweet potato + high oleic peanut combination diet had the least ($P<.0001$). The apparent nitrogen retention for the hens fed the sweet potato treatment significantly lower ($P=0.0004$) than all other treatments. All other treatments analyzed resulted in comparable results and were not different between treatments. The sweet potato + high-oleic peanut diet had higher apparent fat digestibility percentages ($P<.0001$) than any of the other treatments tested. Control-1 had the lowest fat digestibility with the high-oleic peanut diet being higher. The sweet potato diet, had significantly less digestible fat percentage than the sweet potato + high-oleic peanut, but was significantly higher than all other

treatments with control-2 being right behind it. The apparent protein digestibility of sweet potato was higher than all treatments and control-2 was the lowest ($P<.0001$). Control-1 and high-oleic peanut treatments were lower than the sweet potato diet but greater than the control-2, however were not significantly different than the sweet potato + high-oleic peanut diet. Feeding high oleic peanuts and sweet potatoes separately have shown to be comparably digestible making them an even more valuable alternative feed ingredient. The sweet potato + high-oleic peanut diet may need to have an added energy source to create optimal metabolizable energy levels.

INTRODUCTION

The search for viable alternative feed ingredients for poultry continues as the prices for conventional feed ingredients like corn and soybean meal rise. Some countries that do not have the means to pay at these prices, utilize ingredients for feeding that they grow locally such as sweet potato meal, cassava meal, and peanut meal. It is important that these feed ingredients can replace corn fully or partially, without having negative effects on performance and production (Ojewola *et al*. 2006). Some research has examined how feeding high-oleic peanuts affects performance of both broilers and laying hens (Toomer *et al*. 2020; Redhead *et al*. 2021), while others have evaluated how feeding sweet potato roots, vines, and peels would affect poultry performance (Afloyan, 2010). Most have only examined the performance and production aspects of these ingredients, but it is also important to understand the nutrient digestibility of these ingredients when fed.

A feeding trial compared a conventional control, a control diet supplemented with 6% oleic acid, and a high-oleic peanut diet to determine nutrient ileal digestibility as well as apparent metabolizable energy (AMEn) when fed to broilers (Toomer *et al*. 2020). They reported that there were no significant treatment differences in apparent ileal fat or apparent protein digestibility between treatments. However, the HOPN treatment group had a significantly higher AMEn than the other two treatments (Toomer *et al*. 2020). In parallel, Redhead *et al*. (2021) reported significantly higher AMEn levels for their high oleic peanut treatment group as compared to the conventional control in a layer feeding trial. The ileal protein digestion was also greater in hens fed the high oleic peanut-containing diets compared to the conventional controls. With both studies reporting higher AMEn when feeding HOPN, this implies that HOPN could be a good energy source to feed to poultry because of how accessible the energy is to the bird.

In a study that examined the AMEn of broilers fed sweet potato cultivar, the apparent metabolizable energy was reported at 15.39 MJ/kg (Glatz, 2007), which was higher than the corn 14.34 MJ/kg (Ravindran *et al*. 1995). Other studies have determined the apparent metabolizable energy for dehydrated sweet potato roots in a broiler feeding trial as 15.9 MJ/kg (FAO, Inra Cirad Afz, 2012-2015). Hence, feeding trials have reported similar levels of metabolizable energy in corn, sweet potato and dehydrated sweet potato roots. However, there are still very few nutrient digestibility poultry feeding trials. Thus, in this study, we aimed to determine the nutrient digestibility of sweetpotato by-products and/or unblanched high oleic peanuts, by analyzing the apparent protein and fat digestibility and the apparent metabolizable energy.

MATERIAL AND METHODS

This trial was conducted in the bird wing of Scott Hall in the Prestage Department of Poultry Science at North Carolina State University. All methods and procedures used for animal research in this digestibility trial were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC #19-761-A). Eighty Shaver laying hens (36-42 weeks) were housed one bird per cage in PVC coated wire cages which were 30.5 cm X 45.7 providing 1393.9 cm^2/hen. One week was allowed for the birds to acclimate to their surroundings before being fed the experimental diets. Hens were randomly assigned to one of five different treatment groups with sixteen replicates per treatment. Birds had access to feed and water *ad libitum* and were on a 14:10 L:D schedule.

To formulate these five experimental treatments, Concept 5 (level 2, version 10) was used. All diets were formulated to be isocaloric (2,928 kcal/kg) and isonitrogenous (19.5% crude protein) with an estimated particle size between 800 and 1000 µm (Table 1). All diets were manufactured with yellow corn and solvent extracted defatted soybean meal. Two controls were used in this study, one a conventional control (Control-1) and the other with added Soy Protein Isolate (ADM, Chicaogo, Illinois, USA). The sweetpotato by-product only diet (SWP) was manufactured using dried Covington sweetpotato by-products added at 4% inclusion. The sweetpotato by-product used in these diets included peelings, skins, and small tubers and were donated from Yamco LLC. (Snow Hill, NC) frozen. These by-products were allowed to thaw at 4 ºC and were then ground using a Buffalo meat grinder. They were dried utilizing blowers at ambient temperatures to obtain a moisture level below 10%. Analysis for nutritional content of the dehydrated, ground Convington sweetpotato by-products were done by ATC Scientific (Little Rock, AR, USA) before formulating and manufacturing the experimental diets. The analysis results for the sweetpotato are as follows: 0.96% crude fat, 11.0% crude protein, 10.4% ash, and 66.8% carbohydrates, with 102ppm B-carotene, and a gross energy of 3447 kcal/kg.

The high oleic peanut only diet (HOPN) was made with 8% unblanched (skin-intact) high-oleic peanuts. The combination diet, SWP + HOPN, used 4% SWP and 4% HOPN. Only aflatoxin-free unblanched peanuts were used in all experimental diets prepared using peanuts. Peanuts were ground using a Roller Mill forming crumbles before they were included in the completed diet. Diets were supplemented with selenium, vitamin, and mineral premixes that were prepared at the North Carolina State University Feed Mill (Raleigh, NC, USA) adhering or surpassing poultry requirements for those premixes. All experimental diets had analysis run and were analyzed by the North Carolina Department of Agriculture and Consumer Services and the Food and Drug Protections Division Laboratory (Raleigh, NC, USA) for aflatoxin and microbiological contaminants. All feed ingredients and feed sampled were without aflatoxin or microbiological contaminants. Diets were analyzed for crude protein and crude fat values by an AOAC-certified lab, ATC Scientific (Little Rock, AR, USA), using AOAC 990.03 and 920.39-1920 standard methods respectively. All diets contained 2% of CELITE (Diatomaceous Earth, Celite Corp, Lompoc, CA) to be an insoluble ash marker in the diets, to evaluate the nutrient digestibility with partial excreta collection (Huang *et al*. 2006).

Determining Ileal Digestibility

Birds were fed experimental treatments for six weeks with ten birds per treatment being randomly selected for excreta collection Excreta samples were collected using catch pans underneath each bird for three days. The crude protein (CP) values of both the feed and excreta samples were analyzed by ATC Scientific (Little Rock, AR, USA), using AOAC 990.03 methods. Using the excreta, the apparent metabolizable energy corrected for nitrogen (AMEn), the apparent nitrogen retention (ANR), as well as the gross energy of the fecal was determined. At the termination of the study, ileal contents from all the birds were sampled by removal of the gut from the Meckel's diverticulum down to the ileal-cecal junction. The ileum contents were then gently expressed into conical tubes. Ileal contents and excreta were dried at 70°C for 48 hours in a drying oven and ground through a 1-mm screen allowing for further analysis. Gross energy of excreta samples were analyzed using an adiabatic oxygen bomb calorimeter (IKA model C5003 connector to compressed oxygen with NESLAB Refrigerated Re-circulator, CFT-

25). Using a pellet press, fecal samples were compacted down and weighed prior to being placed in a metal thimble with IKA brand 50J cotton twist added for combustion and then placed in the combustion container. The combustion container was sealed, and the contents were combusted. The acid insoluble ash (AIA) of the feed, ileal contents, and excreta samples were analyzed using modified methodology of (Vogtmann *et al*. 1975).

Two grams of each sample was boiled in 25mL of HCl, washed with DI water, and then filtered through ash-less filter paper. The filter paper and residue were placed in pre-weighed crucibles and placed in a muffle furnace at 600°C for 6 hours. Samples were allowed to cool, then were weighed.

$$AME_n = GE_{feed} – (GE_{faecal} \text{ x } AIA_{feed} / AIA_{faecal}) – (8.22 \text{ x } CP_{feacal}/6.25)$$

With:

AME_n = Apparent Metabolizable Energy, corrected for nitrogen

GE = Gross Energy (Bomb Calorimeter)

AIA = Acid Insoluble Ash Recovery

$$ANR = 100 \text{ x } (1-((AIA_{feed} / AIA_{faecal}) \text{ x } (CP_{faecal}/CP_{feed}))$$

With:

ANR = Apparent nitrogen retention

CP = Crude protein

Digestibility % = 100 * (1-((z ileum % / AIA ileum %) / (z diet % / AIA diet %)))

With:

z = one of the measure elements such as protein, fat, etc.

AIA = Acid Insoluble Ash recovery

Total fat content was determined using Soxhlet extraction. Two grams of ground samples of the ieal contents were weighed and placed on filter paper. Samples were then placed in Whatman Cellulose extraction thimbles (26mm x 60mm) and placed into the Sochtec System HT 6 1043 extraction unit Foss Tecator. One hundred milliliter metal canisters were labeled, weighed and approximately 50mL of diethyl ether was added. These canisters were sealed underneath the thimbles and were boiled at 60°C for 40 minutes. Samples were then rinsed for another 40 minutes with the valves open, and another 20 minutes with the valves closed which allowed for ether collection. Ether that was collected in the metal canisters was weighed and recorded.

Statistical Analysis

Each bird served as an experimental unit and all analysis was done using JMP SAS statistical software (version 9.0) for significance by one-way analysis of variance (ANOVA) with a $P<0.05$ being the level of significance. If ANOVA results were significant, a Tukey's multiple comparisons t-test was run to compare the means of the treatments.

RESULTS

The control-1 and HOPN treatment groups had the greatest apparent metabolizable energy (nitrogen corrects), while the SWP + HOPN treatment group had the lowest values when calculated ($P<0.0001$). As shown in Figure 1, control-2 and sweet potato diets are not significantly different from each other in terms of apparent metabolizable energy levels. When the apparent nitrogen retention was analyzed, all treatments had similar apparent nitrogen retention with the exception of the sweetpotato (SWP) treatment group (Figure 2, $P=0.0004$). The apparent ileal fat digestibility of sweetpotato + high-oleic peanut treatment group relative to the other treatment gourps.

As shown in Figure 3, control-1 treatment group had the lowest fat digestibility with high-oleic peanut being second lowest (*P*<0.0001). The sweet potato only diet has less apparent digestible fat than the sweet potato + high oleic peanut, but greater digestible fat than control-2. The apparent ileal protein digestibility was significantly higher in the sweetpotato treatment group relative to the other treatments at 51% (Figure 4, *P*<0.0001). While the control-1 and high-oleic peanut treatments had significantly lower apparent ileal protein digestibility as compared to the sweetpotato treatment group roughly 36% each; the protein digestibility for both tretaments were higher than the control-1 treatment. The apparent ileal protein digestibility for the sweet potato + high-oleic peanut combination treatment was not different from the either the control-1, high-oleic peanut, or control-2 treatment groups with an apparent protein digestibility at 34% (*P*<0.0001).

DISCUSSION

Limited research has been conducted on the nutrient digestibility of sweetpotatoes, sweetpotato by-products, or high-oleic peanuts. Previous studies by Toomer *et al.* (2020) demonstrated in broilers no significant differences in apparent ileal fat or protein digestibility between the HOPN and control treatment groups. However, the findings here showed that the apparent metabolizable energy was the same for both the conventional control and high-oleic peanut treatments. These results imply that both control-1 and high-oleic peanuts have higher availability of energy compared to the other experimental treatments. The sweet potato + high-oleic peanut diet had the lowest apparent metabolizable energy indicating that this diet had the least amount of dietary energy available for digestion. Glatz (2007) reported apparent metabolizable energy values of sweet potato cultivar fed to broilers at 15.39 MJ.kg (3678 cal/g) which is greater than the that of the sweetpotato by-products used in this feeding trial and might be due to the difference in energy utilization of broilers and layers. To date, no research has shown the effects of feeding sweetpotatoes or high-oleic peanuts on apparent nitrogen percentages. The sweetpotato treatment group had the lowest apparent nitrogen retention, suggesting that these birds had a lower ability to retain, or obtain, the nitrogen from this diet to utilize. All other diets had similar apparent nitrogen retentions.

Control-1 has the least apparent fat digestibility implying that dietary fat in this treatment groups was not as readily accessible to the hens fed compared to the other four diets. The sweetpotato + high-oleic peanut diet had the most available dietary fat for digestion and utilization with the sweetpotato treatment group being slightly lower. The high-oleic peanut treatment had better fat digestibility than the conventional control treatment group (Control-1). The sweetpotato treatment had the greatest apparent protein digestibility percentages, which implicates that the protein in this diet was more easily digestible for the hens than the other diets were. The control-1 and high-oleic peanut protein digestibility were next best at having protein available for digestion, which parallels findings of Toomer *et al*. (2020). The control-2 treatment had the lowest availability of protein in comparison to all other treatments. Overall, feeding only sweetpotato by-products results in high protein and fat digestibility and an intermediate metabolizable energy. Feeding high-oleic peanuts also had great benefits, however the sweetpotato + high-oleic peanut diet resulted in improved dieatary fat digestibility, an intermediate dietary protein digestibility, but a limited amount of apparent metabolizable energy compared to the other treatment groups. From these findings, feeding either high-oleic peanuts and sweetpotato by-products separately would be good alternatives for laying hens due to the high levels of nutrient digestibility, and the sweetpotato + high-oleic peanut combination treatment had modest levels of nutrient fat and protein digestibility, and would require supplementation with another dietary energy source.

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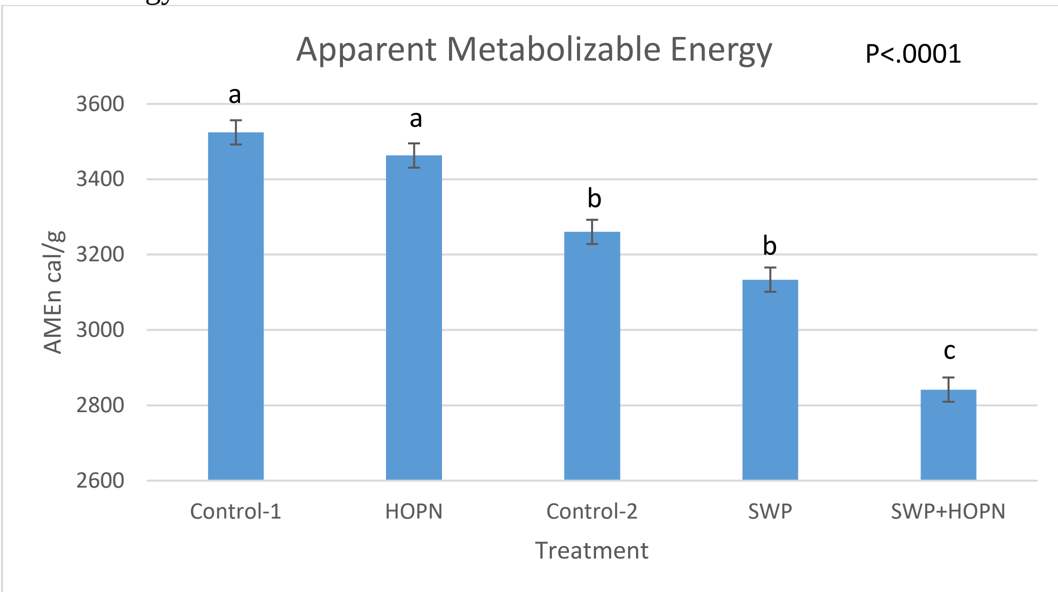
Table 1. Composition of formulated experimental laying hen diets.

	Treatments[1]				
	Control-1	HOPN	SWP	SWP + HOPN	Control-2
Feed Ingredient			%		
Yellow Corn	51.8	51.8	46.5	46.7	54.2
Soybean Meal	32.2	27.8	32.2	30.0	28.8
Calcium Carbonate	9.6	8.9	9.6	9.4	9.6
Dicalcium Phosphate	1.8	2.6	1.8	2.0	1.8
SWP	0.0	0.0	4.0	4.0	0.0
HOPN	0.0	8.0	0.0	4.0	0.0
Sodium Chloride	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.0	0.08	0.0	0.03	0.02
DL-Methionine	0.18	0.2	0.19	0.19	0.17
ADM Soy Protein[2]	0.0	0.0	0.0	0.0	1.6
Soybean Oil	3.7	0.0	5.1	3.1	3.1
Santoquin®[3]	0.05	0.05	0.05	0.05	0.05
Choline Chloride	0.05	0.05	0.05	0.05	0.05
Mineral Premix[4]	0.2	0.2	0.2	2.0	2.0
Vitamin Premix[5]	0.05	0.05	0.05	0.05	0.05
Selenium Premix[6]	0.05	0.05	0.05	0.05	0.05
ME (kcal/kg)	2922	2922	2922	2922	2922

[1]Five experimental isonitrogenous (19.5% crude protein) diets were formulated: Control-1=conventional diet containing defatted soybean meal + corn; HOPN=diet containing 8% unblanched (skin intact) high
oleic peanuts + defatted soybean meal + corn; SWP=diet containing 4% sweetpotato by-products (peelings, small tubers) + defatted soybean meal +corn.
SWP + HOPN=diet of 4% sweetpotato by-products + 4% HOPN + defatted soybean meal + yellow corn; Control-2=diet containing defatted soybean meal + corn + soy protein isolate. Aflatoxin-free peanuts were used in the preparation of all peanut-containing diets.
[2]Soy Protein Isolate provides per kg of diet: crude protein, 14.4 mg; crude fat, 0.64 mg; crude fiber, 0.16 mg; calcium, 0.02 mg; available phosphorus, 0.04 mg; methionine, 0.224 mg; cysteine, 0.192 mg; lysine, 1.01 mg; and threonine, 0.592 mg.
[3]Santoquin®= Feed antioxidant and preservative to prevent fat oxidation in stored feed (Novus International, St. Charles, MO, USA).
[4]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

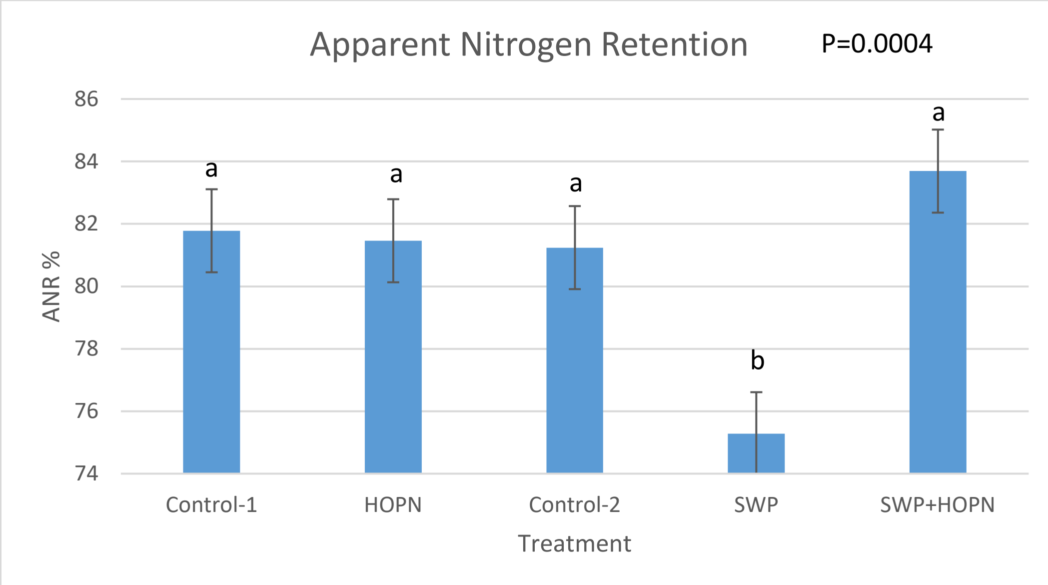
[5]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B_{12}, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B_6, 55 mg niacin, and 1.1 mg folic acid.
[6]Selenium premix=1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.
ME=metabolizable energy
[4]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.
[5]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B_{12}, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B_6, 55 mg niacin, and 1.1 mg folic acid.
[6]Selenium premix=1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.
ME=metabolizable energy

Figure 1. The effect of feeding sweet potato and/or high oleic peanuts on apparent metabolizable energy[1].



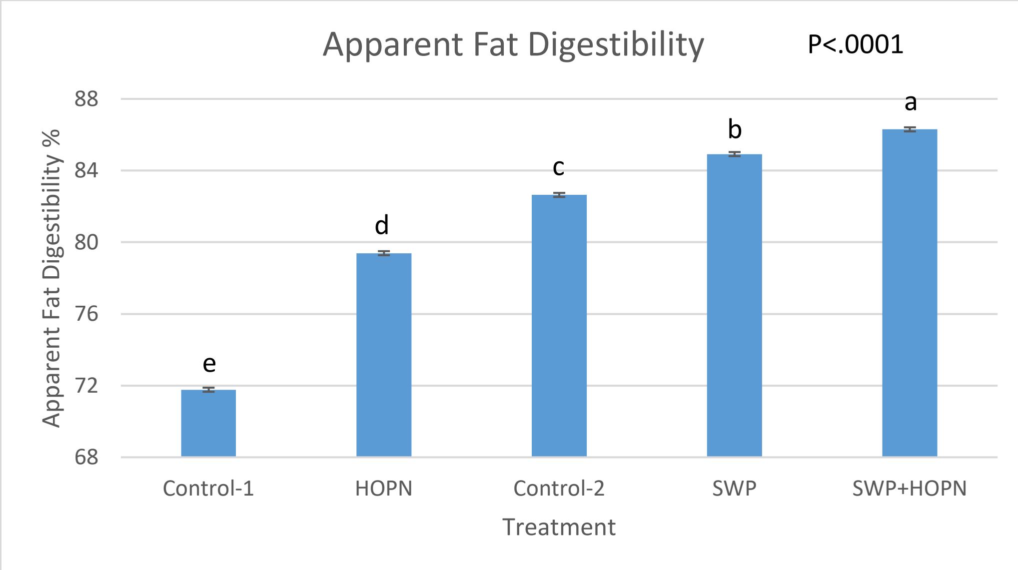
[1]Five isonitrogenous, isocaloric treatments with a 2% inclusion of Celite were fed, a corn-soybean control (control-1), a corn-soybean and soy protein isolate control (control-2), an 8% inclusion of high-oleic peanuts (HOPN), a 4% inclusion of sweet potato (SWP), and a 4% inclusion of sweet potato + 4% inclusion of high-oleic peanuts (HOPN), to eighty Shave laying hens for 6 wks. Each bar graphs represents the average ± SE. $AME_n = GE_{feed} - (GE_{fecal} *$ acid-insoluble ash $recovery_{feed}) - (8.22 *$ crude $protein_{fecal}/6.25)$. [a,b,c]Bar graphs with different superscripts are significantly different.

Figure 2. The effect of feeding sweet potato and/or high oleic peanuts on apparent nitrogen retention[1].



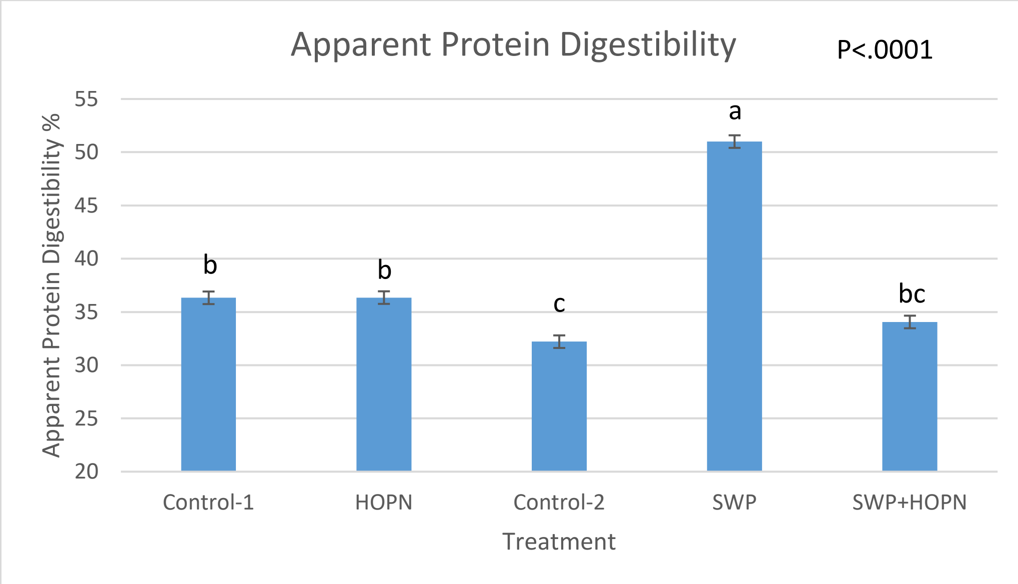
[1]Five isonitrogenous, isocaloric treatments with a 2% inclusion of Celite were fed, a corn-soybean control (control-1), a corn-soybean and soy protein isolate control (control-2), an 8% inclusion of high-oleic peanuts (HOPN), a 4% inclusion of sweet potato (SWP), and a 4% inclusion of sweet potato + 4% inclusion of high-oleic peanuts (HOPN), to eighty Shave laying hens for 6 wks. Each bar graphs represents the average ± SE. ANR = 100 * (1-((acid insoluble ash_{feed} / acid insoluble ash_{fecal}) * (crude $protein_{fecal}$ / crude $protein_{feed}$)). [a,b]Bar graphs with different superscripts are significantly different.

Figure 3. The effect of feeding sweet potato and/or high oleic peanuts on apparent fat digestibility[1].



[1]Five isonitrogenous, isocaloric treatments with a 2% inclusion of Celite were fed, a corn-soybean control (control-1), a corn-soybean and soy protein isolate control (control-2), an 8% inclusion of high-oleic peanuts (HOPN), a 4% inclusion of sweet potato (SWP), and a 4% inclusion of sweet potato + 4% inclusion of high-oleic peanuts (HOPN), to eighty Shave laying hens for 6 wks. Each bar graphs represents the average ± SE. AFD % = 100 * (1-((crude fat_{ileum}/acid insoluble ash_{ileum}) / (crude fat_{feed} / % $Celite_{feed}$))). [a,b,c,d,e]Bar graphs with different superscripts are significantly different.

Figure 4. The effect of feeding sweet potato and/or high oleic peanuts on apparent protein digestibility[1].



[1]Five isonitrogenous, isocaloric treatments with a 2% inclusion of Celite were fed, a corn-soybean control (control-1), a corn-soybean and soy protein isolate control (control-2), an 8% inclusion of high-oleic peanuts (HOPN), a 4% inclusion of sweet potato (SWP), and a 4% inclusion of sweet potato + 4% inclusion of high-oleic peanuts (HOPN), to eighty Shave laying hens for 6 wks. Each bar graphs represents the average ± SE. APD % = 100 * (1-((crude $\text{protein}_{\text{ileum}}$/acid insoluble $\text{ash}_{\text{ileum}}$) / (crude $\text{protein}_{\text{feed}}$ / % $\text{Celite}_{\text{feed}}$))). [a,b,c,]Bar graphs with different superscripts are significantly different.

CHAPTER 5

The Effect of Feeding Sweet Potato and/or High-Oleic Peanut Diet on Layer Performance and the Quality and Chemistry of Eggs Produced

ABSTRACT

Demand for high energy poultry feed ingredients has increased with demands for human consumption, and ethanol production in the US. This competition has increased feed cost, making it crucial for the poultry industry to find economical alternative feed ingredients. Past research has implicated sweet potato by-products (SWP) as an acceptable ingredient in poultry feeds. Our research aim was to examine the effect of high oleic peanut (HOPN) and SWP on hen production and egg quality. Five isocaloric, isonitrogenous diets were used in this study, a conventional control (C1), a soy protein-isolate control (C2), 4% SWP diet, an 8% HOPN, and a 4% HOPN+4% SWP. Seven hundred twenty, Shaver laying hens were randomly assigned to one of the five treatments, with four replicates of thirty-six birds per treatment for 6 weeks. Body and feed weights were recorded bi-weekly. Eggs were tabulated daily and totaled bi-weekly. Thirty eggs per treatment replicate were collected bi-weekly for egg quality analysis and 16 eggs per treatment were used for chemical analysis. All data was analyzed using a 1-way analysis of variance at $p<0.05$ significance. There were no treatment differences in egg or body weights. Hens fed the C2 and HOPN diets produced significantly more eggs ($p<0.01$), relative to the other treatments. The feed conversion ratio (eggs g/feed g) was improved in C1 and C2 hens, relative to the other treatments ($p<0.0001$), while similar between the other treatments. At week 4 and 6, eggs produced from hens fed the SWP treatment had increased egg yolk color relative to the HOPN and SWP + HOPN treatments ($p<0.01$). Stearic fatty acid levels were lowest in eggs produced from hens fed the HOPN and SWP + HOPN treatment groups ($p<0.0001$). These

results suggest that egg yolk color may be enhanced with feeding laying hens a SWP supplemented diet relative to a HOPN-containing diet.

INTRODUCTION

As the poultry industry continues to grow, competition for high energy feed ingredients, like corn, has caused an increase in feed costs (Mmereole, 2008). Corn is used in many ways including high levels of human consumption, livestock consumption, and ethanol production in the United States (Kreutzer, 2012). The increase in feed costs can cause a decrease in poultry production as well as an increase in cost for the consumer. Some countries that do not have food security have shut down some of their broiler facilities because of the increased feed costs (Sakib, N., Sultana, F., Howlider, M.A.R, Rana, M.S., 2014). Because of the shortage of high energy grains, such as corn, it has become imperative to find alternative feed ingredients that can replace these grains without reducing the performance or production or increasing costs. Past research has looked at sweet potato and how it effects the performance and production of broilers and layers. Sweet potato is thought to be a good candidate because it has similar metabolizable energy levels as corn (Woolfe, 1992). Sweetpotato storage roots were found to be good sources of carbohydrates, vitamins, and β-carotene (Bromfield & Bovell-Benjamin, 2002; Byamukama, Gibson, Aritua, & Adipala, 2004). Research conducted with layers determined that peeled sweet potato meal can replace 75% of corn in the diet without adversely affecting hen performance (Agwunobi, 1993). Another study demonstrated that 100% replacement of corn in diet with sun dried sweet potato meal did not adversely affect egg production, egg weight, feed intake or egg shell thickness, Haugh unit, or total feed consumed/dozen eggs in a 12-week layer trial (Khan, 2017).. A trial conducted by Hassan and Abd-El Galil (2013) looked at different levels of sun-dried sweet potato peel waste (0, 15, 20, 25, 30%) in another 12-week layer trial and they determined that up to 25% of the sweet potato peel waste could replace corn in the diet without negatively affecting layer performance or egg quality. However, final body weights declined with increasing levels of sweet potato peel waste.

North Carolina has ranked as the number 1 sweet potato producing state in the U.S. since 1971, providing nearly 60% of the U.S. annual supply (North Carolina Sweet Potato Commission, 2022). Sweet potato waste by-products are generated annually in the form of culled whole sweet potatoes or remnants from food manufacture processing. Culled whole sweet potatoes are rejected due to damage during harvest, transportation or storage, inferior size or weight, or damage from insects, or mold, while sweet potato peels and/or chunks from whole sweet potato flesh are generated during processing (Thibodeau, Poore, & Rogers, 2002), producing approximately 7,000 metric tons of sweet potato waste by-products (Akoetey, Britain, & Morawicki, 2017) annually worldwide.

North Carolina also ranks within the top 6 peanut producing states within the United States: Georgia, Florida, Alabama, Texas, North Carolina, and South Carolina (National Peanut Board. 2021). In our previous layer feeding trials, we demonstrated the efficacious use of unblanched high-oleic peanuts as a suitable alternate layer feed ingredient to enrich the eggs produced with unsaturated fats, β-carotene, and enhanced yolk color (Toomer et al. 2019, 2021a). Nevertheless, while body weights and feed consumption of hens fed a 24% unblanched high-oleic peanut-containing diet was similar to that of hens fed a control diet containing soy protein isolate, hens fed the high-oleic peanut diet produced significantly less eggs (Toomer et al. 2021b). Therefore, in this study we aimed to compare layer performance (body weights, feed intake and egg production) between a conventional control layer diet of defatted soybean meal and yellow corn to a control diet containing defatted soybean meal, yellow corn and soy protein isolate. Also, we aim to determine the effect of sweet potato by-products on layer performance and the quality and chemistry of the eggs produced. Moreover, we aimed to determine the effects of feeding one-third of the previous inclusion level of unblanched high-oleic peanuts in the diet (8%) of layers to determine the effects on layer production performance, egg chemistry and quality.

MATERIALS AND METHODS

Experimental Design, Animal Husbandry and Dietary Treatments

This study was conducted at the North Carolina Department of Agriculture and Consumer Services Piedmont Research Station (Salisbury, NC, USA). Prior to the onset of this study, all experimental protocols and procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee (IACUC #19-761-A, approved 11/27/2019, expires 11/27/2022). Seven hundred and twenty Shaver laying hens (28 to 34 weeks of age) were randomly assigned to one of the four dietary treatments (144 hens per treatment), with four replicates of thirty-six birds each. . Hens were housed in two Conventional Tri-Deck Stacked Layer Cage Systems with 66.04 x 121.92 cm^2 (26 x 48 in^2) per cage, with 18 birds per cage allowing a space of 175.26 cm^2 per hen. Each cage unit consisted of two rows (upper and lower) with a treatment replicate of 36 hens on each row, for a total of 4 replicates per treatment. The study was conducted in a standard height, windowless enclosed ventilated house.

Throughout the feeding trial, birds were provided 14 L:10 D and feed and water *ad libitum* for 6-weeks. Pen body and feed weights were recorded bi-weekly. Shell eggs were collected and enumerated daily from each pen and replicate and totaled each week. Total number of eggs produced per replicate for each treatment was calculated for the total 6 week feeding trial. The average feed conversion ratio (FCR) was calculated as total feed consumed over the 6-week feeding trial (kg)/total dozens of eggs.

Five experimental diets were formulated in Concept 5 (level 2, version 10.0) to be isocaloric (2,922 kcal/kg) and isonitrogenous (19.5% crude protein) with an estimated particle size between 800 and 1000 µm (Table 1). All experimental diets were prepared with yellow corn and solvent extracted defatted soybean meal. For comparison two experimental control diets were prepared with (Control-2) and without (Control-1) Soy Protein Isolate (ADM, Chicago, Illinois, USA). The sweet potato by-product containing diet (SWP) was prepared using 4% dried Covington sweetpotato by-products + solvent extracted defatted soybean meal + yellow corn. Covington sweetpotato peelings, skins, and small tubers were donated from Yamco, LLC. (Snow Hill, NC) frozen. These sweet potato by-products were thawed at 4ºC and ground using a Buffalo meat grinder and dried to a moisture level below 10% using blowers at ambient temperatures during the summer months. The nutritional content for dehydrated ground Covington sweetpotato by-products was analyzed by ATC Scientific (Little Rock, AR, USA) prior to formulation and preparation of the experimental diets: 0.96% crude fat, 11.0% crude protein, 10.4% ash, 66.8% carbohydrates, 102 ppm β-carotene, gross energy 3447 kcal/kg.

A high oleic peanut experimental diet (HOPN) was prepared using 8% unblanched (skin intact) high-oleic peanuts + solvent extracted defatted soybean meal + yellow corn. An additional experimental diet was prepared using 4% SWP + 4% HOPN diet for comparison. Aflatoxin-free unblanched peanuts were used in all peanut-containing experimental diets and crushed using a Roller Mill to form crumbles, prior to inclusion in the finished diets. Each experimental diets were supplemented with vitamin, mineral, and selenium premixes manufactured at the NC State University Feed Mill (Raleigh, NC, USA) to meet and/or exceed poultry requirements for vitamins, minerals, and selenium. All experimental diets were analyzed by the North Carolina Department of Agriculture and Consumer Services and the Food and Drug Protection Division Laboratory (Raleigh, NC, USA) for aflatoxin and microbiological contaminants. All feed ingredients and feed samples were verified to be free of microbiological contaminants.

Association of Official Analytical Chemists (AOAC)-approved methods for nuts and seeds with crude fat determination using Gravimetric methods for nuts-AOAC 948.22, protein determination using Kjeldahl method for nuts-AOAC 950.48, mineral determination by elemental analysis of mineral by atomic absorption spectroscopy, carbohydrates were determined using standard colorimetric assay determination and spectroscopy, enzymatic-gravimetric methods were used for carbohydrate determination AOAC 991.43, standard bomb calorimetry methods were used to determine gross energy, and β-carotene was determined using standard high-performance liquid chromatography and spectrophotometry methods.

Egg Quality and Grading

Egg quality was conducted bi-weekly (0, 2, 4, 6) using a 120 sub-sample of eggs randomly selected from each treatment (6 eggs/replicate) in the Egg Quality Lab, Prestage Department Poultry Science, NC State University (Raleigh, NC, USA). Egg quality parameters measured included shell strength, vitelline membrane elasticity (VME), vitelline membrane hardness (VMH), vitelline membrane work of penetration (VMW), egg weight, albumen height, Haugh unit (HU), yolk color, shell color and shell thickness. Eggshell strength was determined using a texture analyzer (TA-HDplus) with a 250 kg load cell measuring in grams of force. The TA-HDplus has a trigger force of 0.02 kg and a testing speed of 1mm/second. Vitelline membrane strength was determined using the TA.XTplus Texture Analyzer (Stable Micro

Systems, Surrey, United Kingdom) with a 1mm blunt probe with a 5-kilogram load cell per the manufacturer's instructions. The trigger force was 0.0001 kg with a 3.2 mm/second testing speed. Haugh Unit and albumen height were analyzed using the TSS QCD System (Technical Services and Supplies, Dunnington, York, UK). HU is calculated using the following calculation=100Log (h-1.7w + 7.6), with h=egg albumen height and w=weight of egg, with values ranging from 0 to 130 and HU scores below 60 for un-fresh eggs (Nematinia, E. and Abdanan Mehdizadeh, S., 2018) Yolk color was also determined using the TSS QCD System yolk color scan. Yolk color scan was calibrated using the DSM Yolk Color Fan that determines the color density from lightest to darkest with a range of 1 to 15 (Vuilleumier, 1969). Shell color was determined using refractometry of black, blue, and red wavelengths combined to provide a score from 83.3% (white) to 0% (black). USDA shell egg grading and sizing were conducted on a 120 sub-sample of eggs randomly selected from each treatment group (30 eggs/replicate) bi-weekly.

β-Carotene, lipid, and fatty acid analysis

At week 0 and week 6, a total of 144 eggs were randomly selected, with 16 eggs per treatment (4 eggs randomly selected per replicate) for lipid content (total cholesterol, crude fat, and fatty acid profile) and β-carotene analysis by ATC Scientific using AOAC approved methods. Each egg sample was mixed for homogeneity in a Whirl-pak® (Millipore Sigma, St. Louis, MO, USA) bag for 30 seconds in a Smasher™ Lab Blender (Weber Scientific, Hamilton, NJ, USA), the homogenous egg sample was pipetted into a 50ml conical tube and frozen at -20°C and stored until analysis within 2 weeks of collection. Frozen homogenous egg samples were shipped on dry ice overnight to vendor for analysis. Total cholesterol, crude fat, and fatty acid analysis was conducted using direct methylation methods, as described by Toomer et al.

(2019). Total cholesterol was measured as mg cholesterol/100 g sample weight (feed or egg), while crude fat was measured as a percentage of gram crude fat/gram sample weight (feed or egg). Fatty acid content was measured as a percentage of gram of fatty acid/gram total lipid content of a sample (feed or egg). Methods used to determine β-carotene content in eggs are detailed in the AOAC 958.05 color of egg yolk method. Egg fat hydrolysis methods were determined using the AOAC method 954.02.

Statistical analysis

Each treatment replicate (36 hens) served as the experimental unit for all variables (body weights, egg weights, feed intake, total dozens of eggs produced, feed conversion ratio). All performance data was evaluated for significance by one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$ using JMP statistical software (version 15.2.1, SAS, Cary, NC, USA). If ANOVA results were significant ($p < 0.05$), a Tukey's multiple comparisons t-test was conducted to compare the mean of each treatment group with the mean of every other treatment at $p < 0.05$ significance level. The individual egg served as the experimental unit for analysis of all egg quality measurements (120 eggs per treatment, 30 eggs/replicate at each time point) and egg chemistry data (16 eggs per treatment, 4 eggs/replicate at each time point of collection) including crude fat, total cholesterol, fatty acid profile, and β-carotene content between the four treatment groups was conducted for significance by one-way analysis of variance (ANOVA) at a significance level of $p<0.05$ using JMP statistical software (version 9.0).

RESULTS

Dietary Treatments and Hen Performance

Chemical analysis of the experimental diets revealed that diets containing the high-oleic peanuts (HOPN, SWP + HOPN) had higher levels of oleic fatty acid and crude fat relative to the other dietary treatments (Table 2). Dietary levels of calcium and phosphorus of all experimental diets were adequate, meeting the National Research Council (National Research Council, 1994) nutrient requirements for laying hens for calcium (≈2.0% of 2900 kcal/kg diet) and phosphorus (≈0.35% of 2900 kcal/kg diet). Moreover, the high-oleic peanut containing diets (HOPN, SWP + HOPN) diets had the lowest levels of palmitic and stearic saturated fatty acids relative to the other treatment groups. As expected, the experimental diets containing high-oleic peanuts (HOPN, SWP + HOPN) had the highest levels of oleic fatty acids relative to the other diets.

There were no significant treatment differences in body weights at any of the bi-weekly time points measured (Table 3). Hens fed the control-2 (containing soy-protein isolate) and HOPN diets produced significantly more eggs ($p<0.01$), relative to the other treatment groups over the 6-week feeding trial (Table 4). Interestingly, in this study egg production was not similar between comparisons of the control groups, with control-1 group producing less eggs in comparison to control-2treatment. Hens fed the SWP + HOPN experimental diet produced the least number of eggs over the 6-week feeding trial.

Hen egg production was only significantly different between hens of the control-2 treatment group relative to the control-1 and SWP treatment groups, with a greater egg production rate in the control-2 treatment group ($p<0.01$). Hens fed the HOPN diet consumed more feed (grams feed/bird/day) relative to hens fed the control-1 and SWP treatment groups over the 6-week feeding trial ($p \leq 0.0001$). There were no significant treatment differences in mortality rates between the treatment groups over the 6-week feeding trial (Table 4). However, the feed conversion ratio (FCR) was higher and thus better for hens fed the control treatment groups (control-1, control-2), relative to the HOPN, and SWP + HOPN ($p=0.0547$) treatment groups. FCR was similar between hens fed the HOPN, SWP and SWP + HOPN treatment groups over the 6-week feeding trial. There were no significant treatment differences in the average egg weights over the 6-week feeding trial.

USDA Grading and Egg Quality

There were no significant treatment differences in the USDA grading or size of eggs sub-sampled bi-weekly, with greater than 96% of all eggs categorized as USDA Grade A eggs (Table 5). More than 86% of all eggs sub-sampled biweekly were large size eggs, with less than 7% extra-large and 2% small sized eggs produced. At the onset of the study (week 0), there were significant treatment differences in vitelline membrane elasticity (VME) and shell thickness egg quality parameters ($p<0.05$) only (Table 6). However, by week 2 of the study, there were significant treatment differences in albumen height, Haugh unit, yolk color, and shell thickness. Albumen height was similar between the control groups (control-1, control-2) and the albumen height of eggs produced from hens fed the control-1 was significantly greater than the albumen height of eggs produced from hens fed the other treatment groups. At week 2, egg Haugh Unit (HU) was also similar between the control groups (control-1, control-2), while HU was statistically similar between the control-2, HOPN and SWP treatments groups. Egg HU was significantly different between the control-1 and SWP + HOPN treatments at week 2 ($p<0.01$), with lower HU in eggs from the SWP + HOPN treatment group in comparison to control-1 eggs. At week 2, egg yolk color was significantly greater in eggs produced from hens fed the SWP treatment group in comparison to eggs produced from hens fed the HOPN and SWP + HOPN treatment groups ($p<0.01$), however egg yolk color was similar between the SWP and control treatment groups (control-1, control-2). Egg shell thickness was significantly different and greater in eggs produced from hens fed the SWP diet in comparison to the eggshell thickness of eggs produced from the HOPN treatment at week 2 ($p<0.05$).

At week 4 and 6 (Figure 1-Week6), eggs produced from hens fed the SWP treatment had increased egg yolk color relative to the HOPN containing treatment groups (HOPN, SWP +HOPN). At week 4 of the feeding trial, eggs produced from hens fed the SWP diet had greater yolk color scores relative to the HOPN and SWP + HOPN treatment groups ($p \leq 0.001$), while egg yolk color was similar between the control (control-1, control-2) and SWP treatment groups. At week 6, eggs produced from hens fed the SWP treatment had significantly greater yolk color than eggs produced from hens fed the SWP + HOPN treatment group only (Table 6), while egg yolk color was similar between the other treatment groups ($p<0.01$).

Egg Chemistry

At the onset of the study (week 0), eggs in the HOPN had significantly greater palmitic acid levels (Table 7), relative to the SWP and SWP + HOPN treatment groups, while levels were similar between the HOPN and control (control-1, control-2) treatment groups ($p \leq 0.001$). Also, at week 0, egg linoleic acid levels were significantly greater in control-1 and HOPN treatment groups, relative to the SWP treatment group ($p \leq 0.01$). There were no significant treatment differences in egg fatty acid, crude fat, total cholesterol, or β-carotene levels at week 0 of the study.

There were significant treatment differences in egg stearic acid ($p<0.0001$), linoleic acid ($p<0.0001$), total omega 3 fatty acids ($p<0.001$), and nervonic acid ($p<0.0001$) levels at week 6 of the feeding trial (Table 7). Similar to our previous studies (Toomer et al., 2019), saturated stearic fatty acid levels were lowest in eggs produced from hens fed the high-oleic peanut containing diets, (HOPN and SWP + HOPN) treatment groups, relative to eggs from the SWP treatment group at week 6. Eggs produced from hens fed the SWP and control-1 diets had significantly higher levels of linoleic acid in comparison to eggs from the HOPN, SWP + HOPN, and control-2 treatment groups at week 6. Interestingly, eggs produced from hens fed the SWP diet had significantly higher omega 3 fatty acid levels compared to the HOPN and control-2 treatment groups. At week 6, nervonic acid levels were significantly higher in eggs produced from hens fed the control-1, SWP, and SWP + HOPN treatment groups in comparison to eggs from the HOPN and control-2 treatment groups (Table 7).

DISCUSSION

In this study hens fed the soy protein containing control diet and the HOPN (8%) diets produced significantly more total dozens of eggs with similar egg weights, in comparison to the other treatments. However, in our previous peanut and peanut by-product layer feeding trials, hens fed the unblanched high-oleic peanut supplemented diet (24%) produced significantly less eggs with reduced egg weights relative to the control group containing soy-protein isolate (Toomer et al. 2021b) Hence, suggesting that lower dietary inclusion levels of unblanched high-oleic peanuts does not alter egg production in layers or egg weights. Interestingly, in this study FCR was similar between both control groups (control-1 and control-2), and was improved relative to the other treatment groups, which parallels our previous feeding trial demonstrating improved FCR in the soy protein isolate containing control group (Toomer et al. 2019).

In parallel to our previous peanut layer feeding trials (Toomer et al. 2019, 2021a, 2021b), there were no major treatment differences on USDA grading or egg quality parameters, apart from egg yolk color in this study. In general egg yolk color score was higher and egg yolks were visibly darker yellow/orange color in eggs produced from hens fed the 4% SWP experimental treatment group relative to the other treatment groups, with exception of the controls. In parallel, Kaya and Yildrum (2011) demonstrated that 4% inclusion of dried sweetpotato tubers and vines in the diet of layers did not alter yolk pigmentation in comparison to conventional control eggs. In contrast, other studies have shown that feeding sweetpotato meal at 50% inclusion in layer diets significantly enhanced yolk pigmentation relative to conventional control eggs (Ladokun et al., 2007). Hence in the future we aim to conduct additional sweetpotato waste by-product layer feeding trials at higher inclusion levels to determine the effect on overall egg production, quality and specifically yolk color.

In our previous feeding trials (Toomer et al. 2019, 2021a), egg yolk color score was significantly higher and visibly darker in eggs produced from hens fed a 24% unblanched high-oleic peanut diet in comparison to the conventional controls, while in this study yolk color was similar between eggs produced from hens fed the control diets (control-1 and control-2), the 4% sweetpotato by-product diet, and the 8% unblanched high-oleic peanut diet at week 6. (Therefore, suggesting that higher dietary inclusion levels (24%) of unblanched high-oleic peanuts rich in unsaturated fatty acids enriches egg yolk color, while lower dietary inclusion levels (8%) do not alter egg yolk color. In the last decade, consumers have shown a preference in egg yolk color (Berkhoff et al., 2020), with egg producers catering to this trend using carotenoid-rich feed additives (Grčević, Kralik,Kralik, &Galović, 2019) to produce dark yellow/orange yolks. However, consumer demand for yolk color is also dependent upon region, with Northern Europeans most desirous of pale-yellow yolks and Southern Europeans closest to Italy preferring only bright orange/red egg yolks, known as "*rosso d'uovo*" (Modern Farmer, 2013).

While stearic saturated fatty acid levels were lowest in eggs from hens fed the HOPN diet, stearic acid levels did not differ significantly from the controls at week 6 of the feeding trial. Unlike our previous feeding trials (Toomer et al., 2019, 2021a, 2021b), oleic acid levels were similar between eggs from each treatment group at week 6. Linoleic unsaturated fatty acid levels were highest in eggs produced from the SWP treatment group, however, there were not significantly different from the controls (control 1) at week 6. Lastly, while there were significant treatment differences in the omega 3 and nervonic acid levels in eggs, the content of these fatty acids in the eggs produced were very low (less than 1.2% nervonic acid and less than 2.1% omega 3 acid) at week 6.

In conclusion, hen body weights, total number of eggs produced, USDA grade, egg size or egg quality (exception yolk color) were not adversely affected by the inclusion of 4% sweetpotato by-products in layer diets with 6 weeks of feeding. Nevertheless, more work needs to be performed to identify optimal inclusion levels of sweetpotato by-products in the diet of layers for ideal performance, egg yolk color and chemistry. More importantly, this study supports agricultural sustainability within North Carolina and the US Southeast with the use of agricultural products and waste by-products that are common within these regions as a value-added feed ingredient for poultry and other livestock.

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Table 1. Composition of formulated experimental laying hen diets.

	Treatments[1]				
	Control-1	HOPN	SWP	SWP + HOPN	Control-2
Feed Ingredient			g/kg DM		
Yellow Corn	518	518	465	467	542
Soybean Meal	322	278	322	300	288
Calcium Carbonate	96	89	96	94	96
Dicalcium Phosphate	18	26	18	20	18
SWP	0.0	0.0	40	40	0.0
HOPN	0.0	80	0.0	40	0.0
Sodium Chloride	2.5	2.5	2.5	2.5	2.5
L-Lysine	0.0	0.8	0.0	0.3	0.2
DL-Methionine	1.8	2.0	1.9	1.9	1.7
ADM Soy Protein[2]	0.0	0.0	0.0	0.0	16
Soybean Oil	37	0.0	51	31	31
Santoquin®[3]	0.5	0.5	0.5	0.5	0.5
Choline Chloride	0.5	0.5	0.5	0.5	0.5
Mineral Premix[4]	20	20	20	20	20
Vitamin Premix[5]	0.5	0.5	0.5	0.5	0.5
Selenium Premix[6]	0.5	0.5	0.5	0.5	0.5
ME (kcal/kg)	2922	2922	2922	2922	2922

[1]Five experimental isonitrogenous (19.5% crude protein) diets were formulated: Control-1=conventional diet containing defatted soybean meal + corn; HOPN=diet containing 8% unblanched (skin intact) high oleic peanuts + defatted soybean meal + corn; SWP=diet containing 4% sweetpotato by-products (peelings, small tubers) + defatted soybean meal +corn. SWP + HOPN=diet of 4% sweetpotato by-products + 4% HOPN + defatted soybean meal + yellow corn; Control-2=diet containing defatted soybean meal + corn + soy protein isolate. Aflatoxin-free peanuts were used in the preparation of all peanut-containing diets.

[2]Soy Protein Isolate provides per kg of diet: crude protein, 14.4 mg; crude fat, 0.64 mg; crude fiber, 0.16 mg; calcium, 0.02 mg; available phosphorus, 0.04 mg; methionine, 0.224 mg; cysteine, 0.192 mg; lysine, 1.01 mg; and threonine, 0.592 mg.

[3]Santoquin®= Feed antioxidant and preservative to prevent fat oxidation in stored feed (Novus International, St. Charles, MO, USA).

[4]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt.

[5]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B_{12}, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B_6, 55 mg niacin, and 1.1 mg folic acid.

[6]Selenium premix=1 mg Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet.

ME=metabolizable energy

Table 2. Chemical analysis of sweet potato and/or peanut-containing layer diets.

	Treatments[1]				
	Control-1	HOPN	SWP	SWP+ HOPN	Control-2
Nutrient	g/kg DM				
Crude Fat[2]	56	81	69	81	52
Calcium	28	33	30	22	34
Phosphorous	6.3	7.6	6.2	5.7	6.7
Palmitic Acid (16:0)*	122	95	111	99	109
Palmitoleic Acid (16:1)*	7.5	6.9	2.0	2.6	3.5
Stearic Acid (18:0)*	43	31	42	36	39
Oleic Acid (18:1)*	230	593	208	356	219
Elaidic Acid (C18:1 trans)*	0.5	0.8	0.3	0.6	0.9
Linoleic Acid (18:2)*	510	198	541	416	533
Linolenic Acid (18:3)*	62	13	71	50	63
GLA*	0.4	0.7	0.4	0.6	0.6

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products.

SWP + HOPN= diet containing defatted soybean meal, corn, 4% dried sweetpotato by-products and 4% unblanched high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. Five dietary treatments were chemically analyzed by AOAC-certified lab, (ATC Scientific, Little Rock, AR, USA) using standard AOAC-approved methods.

[2]Crude Fat content = g crude fat/g total sample weight * 100,

*Fatty acid content = g of fatty acid/g total lipid content * 100.

GLA=Homo-gamma (ɣ)-linolenic acid (18:3n-6).

Table 3. Body weights of hens fed a sweet potato and/or peanut-containing diet for 6-weeks.

	Treatments[1]						
	Control-1	HOPN	SWP	SWP + HOPN	Control-2	SEM	*p*-value*
			(kg)				
Week 0	1.64	1.64	1.67	1.67	1.62	0.02	0.07
Week 2	1.57	1.58	1.55	1.59	1.57	0.03	0.75
Week 4	1.58	1.66	1.65	1.66	1.62	0.05	0.47
Week 6	1.61	1.64	1.65	1.62	1.64	0.04	0.85

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products.

SWP + HOPN= diet containing defatted soybean meal, corn, 4% dried sweetpotato by-products and 4% unblanched high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. 720 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 5 treatments with 4 replicates/treatment and provided feed and water *ad libitum* for 6-weeks. Body weights were recorded bi-weekly for each pen (18 hens per pen). Each value represents the replicate (36 hens) mean ± the standard error. *p-value=statistically significant differences $p<0.05$ by analysis of variance (ANOVA).

Table 4. Production performance of hens fed a sweet potato and/or peanut-containing diet.

Treatments[1]	Total Dozen Eggs Produced	Hen Day Production[2] (%)	Feed Consumed[3] (g/bird/day)	Mortality[4] (%)	Feed Conversion Ratio[5] (egg grams/feed grams)	Average Egg Weight[6] (g)
Control-1	120^{b}	92.9^{b}	96.4^{c}	1.74	0.560^{a}	58.1
HOPN	126^{a}	95.9^{ab}	106.2^{a}	0.00	0.526^{b}	58.5
SWP	119^{bc}	92.0^{b}	101.1^{bc}	1.04	0.539^{ab}	58.2
SWP + HOPN	117^{c}	94.1^{ab}	103.2^{ab}	3.13	0.530^{b}	58.2
Control-2	128^{a}	97.9^{a}	102.3^{ab}	0.00	0.559^{a}	58.6
SEM	1.90	1.13	1.29	0.89	0.0097	0.405
p-value	0.006	0.0055	0.0001	0.0909	0.0547	0.349

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products; SWP + HOPN= diet containing defatted soybean meal, corn, 4% dried sweetpotato by-products and 4% unblanched high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. 720 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 5 treatments with 4 replicates/treatment, 36 birds/replicate and provided feed and water *ad libitum* for 6-weeks. Eggs were collected daily and enumerated and weighed weekly from each replicate pen. Feed intake was calculated weekly for each pen (≈18 hens/pen) per bird.
[2]Hen Day Production= total number of eggs laid for the 6-week feeding trial/total number of egg-producing birds
[3]Total feed consumed over 6-week feeding trial=total grams feed consumed per treatment/144 birds/42 days.
[4] Mortality(%)=total # deaths/ number of live chickens per treatment *100.
[5]Feed conversion ratio (FCR)= total grams egg weights for each treatment/g total feed consumed over the 6-week feeding trial.
Each value represents the mean ± the standard error.
[6]Average Egg Weight=egg weights represent the mean ± standard error of 120 sub-sample of eggs collected bi-weekly with 30 eggs randomly selected from each treatment replicate.
**p*-value=statistically significant differences $p<0.05$ by analysis of variance (ANOVA).

Table 5. USDA grading of eggs produced from hens fed a sweet potato and/or peanut-containing diet[1].

Treatments	Grade A	Grade B	Cracks	Ex-Large	Large	Medium	Small
				Percent (%)[§]			
Control-1	96.5	1.88	1.67	5.83	89.0	4.38	0.83
HOPN	96.3	3.13	0.63	4.17	90.0	5.42	0.42
SWP	97.9	0.63	1.46	7.32	86.8	4.39	1.46
SWP + HOPN	96.0	1.88	2.08	4.17	90.6	4.12	0.63
Control-2	98.1	0	1.88	5.00	90.8	3.96	0.21
SEM	1.65	1.65	0.562	2.05	1.61	1.44	0.42
p-value*	0.837	0.705	0.437	0.795	0.423	0.959	0.311

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products.
SWP + HOPN= diet containing defatted soybean meal, corn, 4% dried sweetpotato by-products and 4% unblanched high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. 720 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 5 treatments with 4 replicates/treatment and provided feed and water *ad libitum* for 6-weeks. Eggs were collected daily and enumerated and weighed weekly for each pen (18 hens housed/pen). Bi-weekly (week 0, 2, 4, 6), a sub-sample of 120 eggs (30 eggs/replicate) per treatment were assessed for USDA grading and sizing for a total 480 eggs.
[§]Percentage of the 480 egg sub-sample per treatment.
**p*-value=statistically significant differences $p<0.05$ by analysis of variance (ANOVA).

Table 6. Quality of eggs produced from hens fed a control of sweet potato and/or peanut-containing diet.

		Treatment[1]					
Wk0	Control-1	HOPN	SWP	SWP+HOPN	Control-2	SEM	*p*-value*
Shell Sth. (g force)	5298	5011	5592	5275	5442	219	0.107
VME (mm)	0.29^{a}	0.27^{ab}	0.25^{b}	0.26^{b}	0.26^{b}	0.01	0.004
VMH (g)	2.18	1.94	2.17	2.02	2.12	0.13	0.289
VMW (g/sec)	1.70	1.48	1.72	1.49	1.68	0.16	0.334
Shell Color (%)	82.3	83.3	82.0	83.8	84.7	1.14	0.130
Albumen Ht. (mm)	8.15	8.49	8.68	7.94	8.45	0.30	0.110
Haugh Unit (HU)	90.9	92.5	94.0	89.5	92.3	1.6	0.060
Yolk Color (1-15)	5.88	6.17	6.29	6.46	6.21	0.21	0.090
Shell Thick (mm)	0.37^{b}	0.38^{b}	0.44^{a}	0.38^{b}	0.44^{a}	0.01	<0.0001
Wk2	Control-1	HOPN	SWP	SWP+HOPN	Control-2	SEM	*p*-value*
Shell Sth.(g force)	5473	5350	4936	5457	5432	301	0.352
VME (mm)	0.235	0.232	0.248	0.235	0.241	0.01	0.477
VMH (g)	2.45	2.40	2.25	2.38	2.22	0.17	0.690
VMW (g/sec)	1.96	1.94	1.78	1.91	1.75	0.19	0.733
Shell Color (%)	83.3	81.6	83.5	83.1	83.2	0.79	0.144
Albumen Ht. (mm)	8.85^{a}	8.47^{b}	8.68^{b}	8.08^{b}	8.73^{ab}	0.25	0.015
Haugh Unit (HU)	94.7^{a}	92.6^{ab}	93.9^{ab}	90.5^{b}	94.4^{a}	1.3	0.007
Yolk Color (1-15)	6.83^{ab}	6.50^{b}	7.13^{a}	6.54^{b}	6.88^{ab}	0.18	0.003
Shell Thick (mm)	0.39^{ab}	0.37^{b}	0.39^{a}	0.39^{ab}	0.39^{ab}	0.01	0.022
Wk4	Control-1	HOPN	SWP	SWP+HOPN	Control-2	SEM	*p*-value*
Shell Sth. (g force)	5466	5582	5629	5336	5554	187	0.550
VME (mm)	0.217	0.222	0.225	0.217	0.226	0.01	0.473
VMH (g)	2.31	2.48	2.29	2.26	2.32	0.15	0.604
VMW (g/sec)	1.91	2.09	1.89	1.84	1.91	0.18	0.691
Shell Color (%)	84.7	85.0	85.6	85.3	85.5	0.45	0.249

		Control-1	HOPN	SWP	SWP+HOPN	Control-2	SEM	*p*-value*
	Albumen Ht. (mm)	8.71	8.61	8.48	8.37	8.43	0.213	0.490
	Haugh Unit (HU)	93.5	93.0	91.6	91.8	92.0	1.21	0.436
	Yolk Color (1-15)	6.96^{ab}	6.67^{b}	7.25^{a}	6.17^{b}	7.08^{ab}	0.15	0.001
	Shell Thick (mm)	0.397	0.396	0.396	0.393	0.393	0.01	0.918
W6		Control-1	HOPN	SWP	SWP+HOPN	Control-2	SEM	*p*-value*
	Shell Sth. (g force)	5252	5164	5487	5508	5282	273	0.649
	VME (mm)	0.214	0.226	0.227	0.222	0.217	0.01	0.531
	VMH (g)	2.29	2.40	2.28	2.45	2.20	0.13	0.343
	VMW (g/sec)	1.89	2.02	1.80	2.06	1.77	0.17	0.317
	Shell Color (%)	83.7	84.1	83.8	83.6	84.3	0.76	0.825
	Albumen Ht. (mm)	8.34	8.26	8.16	8.48	8.33	0.23	0.740
	Haugh Unit (HU)	91.5	90.9	90.0	91.9	91.5	1.18	0.503
	Yolk Color (1-15)	6.92^{a}	6.50^{ab}	7.00^{a}	6.33^{b}	6.79^{ab}	0.19	0.003
	Shell Thick (mm)	0.394	0.383	0.383	0.382	0.388	0.01	0.403

[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products.
SWP + HOPN= diet containing defatted soybean meal, corn, 4% dried sweetpotato by-products and 4% unblanched high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. 720 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 5 treatments with 4 replicates/treatment and provided feed and water *ad libitum* for 6-weeks. Bi-weekly at 120 sub-sample of eggs were collected from each treatment group for quality assessment using Technical Services and Supplies QCD system, with calibration with the DSM Color Fan for yolk color. Yolk color=index 1-15 (lightest to darkest color intensity). Shell Sth=shell strength; VME=vitelline membrane elasticity; VMH=vitelline membrane hardness; VMW=vitelline membrane work of penetration; Albumen Ht.=albumen height; Shell Thick=shell thickness. Each value represents the bi-weekly average ± the standard error with 120 eggs/treatment. **p*-value=statistically significant differences $p<0.05$ by analysis of variance (ANOVA).
[a,b]Means within the same column lacking a common superscript differ significantly ($p<0.05$).

Table 7. The β-carotene, lipid and fatty acid analysis of eggs produced from hens fed a sweet potato and/or peanut-containing diet.

	Treatments[1]						
	Control-1	HOPN	SWP	SWP + HOPN	Control-2	SEM	*p*-value
Wk0							
Crude Fat %[2]	5.2	4.3	5.4	5.5	6.4	0.78	0.169
Palmitic % (16:0)*	22.7^{ab}	23.2^{a}	21.2^{b}	21.6^{b}	22.2^{ab}	0.47	0.001
Stearic % (18:0)*	9.2	9.6	9.1	9.0	9.3	0.23	0.142
Oleic % (18:1)*	28.4	29.6	27.5	28.1	28.8	0.68	0.33
Elaidic%(C18:1trans)*	0.18	0.09	0.23	0.21	0.10	0.06	0.09
Linoleic % (18:2)*	24.3^{a}	24.5^{a}	22.5^{b}	23.4^{ab}	23.9^{ab}	0.57	0.01
Omega 3 % (18:3)*	1.8	1.6	1.9	2.0	1.7	0.18	0.10
Nervonic % (24:1)*	1.0	1.1	1.1	1.1	1.2	0.04	0.0483
β-carotene (ppm)	2.20	1.99	1.92	1.83	2.0	0.26	0.70
Cholesterol (mg/100g)	192	175	184	191	253	36	0.27
Wk6							
Crude Fat %[2]	6.1	7.1	5.7	6.6	5.8	0.87	0.46
Palmitic % (16:0)*	21.4	21.2	21.0	20.6	21.8	0.50	0.15
Stearic % (18:0)*	8.6^{ab}	7.0^{b}	9.0^{a}	7.9^{b}	8.4^{ab}	0.29	<0.0001
Oleic % (18:1)*	30.3	37.6	28.2	34.4	37.2	5.0	0.27
Elaidic%(C18:1trans)*	0.16	6.8	0.16	0.14	0.11	4.6	0.47
Linoleic % (18:2)*	20.4^{ab}	11.8^{c}	23.7^{a}	19.2^{b}	17.4^{b}	1.4	<0.0001
Omega 3 % (18:3)*	1.8^{ab}	1.0^{c}	2.0^{a}	1.6^{abc}	1.2^{bc}	0.23	0.0008
Nervonic % (24:1)*	1.1^{a}	0.73^{c}	1.1^{a}	1.0^{ab}	0.88^{b}	0.05	<0.0001
β-carotene (ppm)	2.4	2.5	2.7	2.7	2.2	0.40	0.65
Cholesterol (mg/100g)	264	244	212	291	252	33	0.21

[1]720 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 5 treatments with 4 replicates/treatment and provided feed and water ad libitum for 6-weeks. 16 eggs/treatment were chemically analyzed at each time point of collection. Each value represents the mean ± standard error.

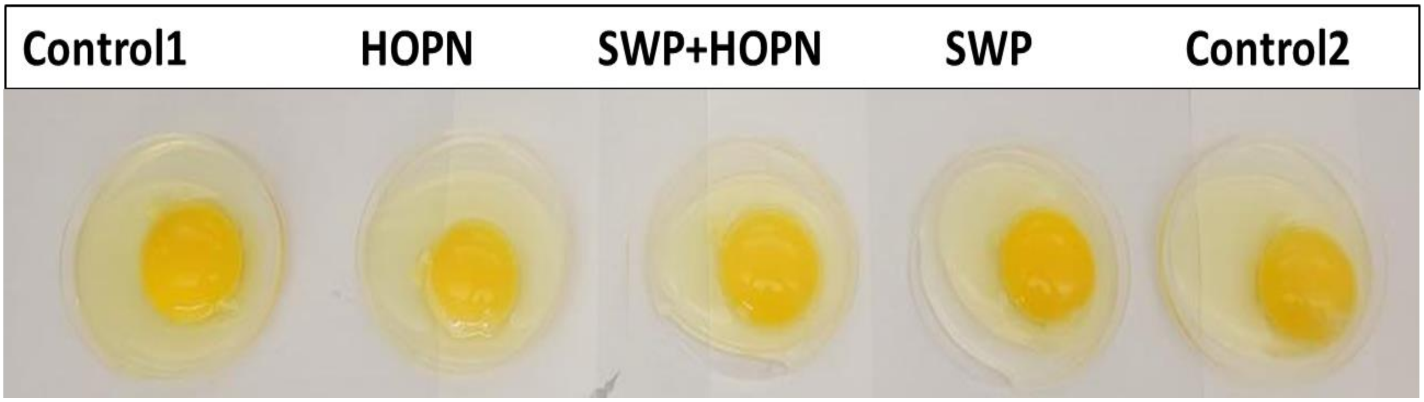
[1]Dietary treatments: Control-1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn and 8% unblanched (skin intact) high oleic peanuts; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products.

SWP + HOPN= diet containing defatted soybean meal, corn, 4% dried sweetpotato by-products and 4% unblanched high-oleic peanuts; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate. Four dietary treatments were chemically analyzed by AOAC-certified lab, (ATC Scientific, Little Rock, AR, USA) using standard AOAC-approved methods.

[2]Crude Fat content = g crude fat/g total sample weight * 100,

*Fatty acid content = g of fatty acid/g total lipid content * 100.

Figure 1. Representative images of yolk color from whole egg samples from each treatment group at week 6 of the feeding trial[1].



[1]720 white Shaver laying hens (28 to 34 weeks of age) were assigned to one of 5 treatments with 4 replicates/treatment and provided feed and water ad libitum for 6-weeks. At 6-weeks, one whole egg was randomly selected for this photograph as a representative of yolk color observations seen on the day of egg processing with 120 eggs per treatment. This image is not representative of any other egg quality parameters measured. Dietary treatments: Control 1=conventional diet containing defatted soybean meal and corn; HOPN=diet containing defatted soybean meal, corn + 8% unblanched (skin intact) high oleic peanuts; SWP + HOPN= diet containing defatted soybean meal, corn + 4% dried sweetpotato by-products + 4% HOPN; SWP=diet containing defatted soybean meal, corn and 4% dried sweet potato by-products; Control-2=diet containing defatted soybean meal, corn, and soy protein isolate.

CHAPTER 6

FINAL CONCLUSIONS

As the demand for ingredients like corn and soybean meal continue to rise, the importance of finding viable alternative feed ingredients increases. Most states that have high poultry production cannot grow enough of these crops to supply the producer's annual needs. Therefore, corn and soybeans must be imported from other states which results in an additional cost. Not only is it important to find alternative feed ingredients that will not hinder poultry performance or product quality but utilizing locally grown feed ingredients to minimize more financial adversities. Other countries that cannot pay the prices for corn have already begun utilizing what they grow to feed their livestock. Some have fed different parts of sweet potatoes to their chickens without seeing reduced production. It had also been shown that peanut parts like the skins can be fed to chickens and have beneficial results. Peanut and sweet potato producers have certain standards their products must meet to be sold for human consumption. These standards include size and grade, and the product can be rejected for not meeting them. Sweet potatoes can also be rejected for damages occurring during processing, transport, or storage. While both of these products are thrown out because they do not meet standards, they maintain their nutritional value and can be utilized for livestock consumption.

The objective of Chapter 2 was to determine the digestibility of whole-in-shell peanuts and high-oleic peanuts when fed to laying hens by measuring apparent metabolizable energy correct for nitrogen (AMEn), apparent nitrogen retention (ANR), apparent fat digestibility, and apparent protein digestibility. The data indicates that C1 and HOPN diets have more dietary energy because of the high levels of AMEn calculated. It also indicates that C2 has more available energy for the birds than the WPS treatment. This implies that the laying hen fed WPS have poorer nitrogen retention compared to the other treatments. The apparent fat digestibility was highest in C2 and HOPN was closely behind, implying that C2 had a higher digestible fat percentage than other treatments and HOPN had the next best fat digestibility. WPS had the lowest apparent protein digestibility indicating that the hen was unable to access the protein in this diet compared to the other ones. C1 had the highest apparent protein digestibility with HOPN was the next best. Overall, this chapter suggests that feeding HOPN is comparable to C1 in all parameters measured. If feeding WPS, it may be beneficial to supplement protein because of the lower protein levels. WPS digestibility should be further researched before they are fully ruled out because they may have greater value in extensive production systems due to the high heat increment.

The objective of chapter three was to determine the effects of feeding WPS and/or HOPN on laying hen performance, production, and egg quality and composition. This was achieved by measuring things like bodyweights, total number of eggs per week, and feed conversion ratio. It was determined that there were significant differences in vitelline membrane elasticity in the beginning of the study for both C1 and C2, however at week 2 and so on there were no differences. This was expected because over time, the vitelline membrane elasticity declines and is heavily dependent on storage conditions. C1 had the highest levels of palmitic saturated fatty while HOPN had the highest levels of palmitoleic acid. Palmitoleic acid can act as an anti-inflammatory to reduce insulin resistance and reduce hypertension. The data shows that WPs had the highest percentages of extra-large eggs compared to the other treatments. Eggs sizes in retail stores are normally determined by the total weight of dozen eggs produced.

Treatment diets also resulted in similar levels of homo-gamma-linoleic (GLA) levels. GLA has been shown to reduce inflammatory biomarkers in many chronic inflammatory disease like rheumatoid arthritis. Yolk color scores were greater in C1 and C2 at week 4 and were higher in week 6 though not statistically different. Egg yolk color is greatly influence by lipid profile of hen diets and sometimes the type and concentration of dietary carotenoids are transferred as are their pigments to the yolks. β-carotene levels were significantly higher in WPS fed hens than other treatments at week 6. The diets however were below the level of detection. Reports have shown that peanut oil has been reported to contain small quantities of β-carotene. Similar body weights, feed intake, FCR, and egg weights were reported between the treatments. This supports that feeding peanuts to laying hens will not hinder performance and product and there is value to adding these ingredients to the diet. It needs to be replicated to mimic commercial egg production to determine the full effects these ingredients have on laying hens over time.

The objective of chapter four was to determine the digestibility of sweet potatoes, high-oleic peanuts, and a sweet potato + high-oleic peanuts when fed to laying hens. This was determined by analyzing the AMEn, ANR, apparent fat digestibility, and apparent protein digestibility. This study showed the C1 and HOPN treatments had higher AMEn compared to the other treatments indicating the diet had more metabolizable energy than the other diets. The SWP + HOPN diet has the least amount of AMEn meaning it has the least amount of energy available for the hens to digest. The SWP treatment had the lowest ANR values so it had the birds fed this diet had the lowest nitrogen retention. C1 had the lowest apparent fat digestibility so the fat from this diet was not as available to the birds. However, SWP + HOPN diet had the highest the most available fat for digestibility with the SWP being only slightly lower. HOPN had better fat digestibility than the control-1 but less than control-2. The sweet potato diet also had the highest apparent protein digestibility so the protein in this diet was presumably more available to the hens than the other diets. C1 and HOPN were the next best protein digestibility with C2 being the lowest.

The objective for chapter 5 was to determine the effects of feeding SWP, HOPN, or a SWP + HOPN diet on laying hen performance, production, and egg quality and composition. Hens that were fed C2 and HOPN had significantly more total dozen eggs with similar egg weights compared to the other treatments. The feed conversion ratio was better for C1 and C2 than the other treatments as well. The data indicates that there are no significant differences when analyzing egg quality, except for yolk color scores. Yolk color scores were higher in SWP treatment hens than others. Some consumers have preference for yolk color believing that darker yolk colors are associated with better nutritional values, however this is dependent on region. Stearic acid levels were lowest in HOPN fed hens and linoleic acid levels.

Body weights, total number of eggs produced, USDA egg grade, egg size, and the majority of egg quality parameters showed no differences in treatments. This indicated that feeding SWP and/or HOPN can be down without decreasing performance or production, and there are some benefits. However, studies must continue to be done to find the best inclusion percentage of SWP to achieve optimal performance and production. This also needs to be run mimicking the commercial egg industry to determine the long term advantages and disadvantages of these alternative feed ingredients. The research in this thesis supports that feeding WPS, HOPN, and/or SWP can be done without decreasing laying hen production or performance. Feeding these ingredients also did not have a negative impact on egg quality. They also are all relatively digestible, however supplementing protein when feeding WPS may be needed and feeding a combination of SWP + HOPN may need supplemented energy.