TRACE ELEMENTS IN LAYING HENS AND BROILER BREEDER HENS

by

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(Under the Direction of Adam J. Davis)

ABSTRACT

With the increasing availability and use of purified nutrients such as amino acids, vitamins and minerals in poultry diets, the overall composition of these diets is becoming more uniform and defined. While this can decrease diet cost and nutrient excesses, it can also lead to the discovery that nutrients once considered as not required actually have a requirement, and that nutrients with established requirements may have higher requirements than previously thought for optimal performance. This phenomenon is especially true with trace element requirements. In the current research the potential for the supplementation of poultry diets with rare earth elements to improve egg production in hens was investigated as was the potential that organic sources of selenium could better alleviate heat stress than an inorganic source of selenium. AZOMITE® is a hydrated sodium calcium aluminosilicate containing macro and trace minerals and rare earth elements. Laying hens were fed a control diet or this diet supplemented with 0.25% Azomite from 54 through 98 weeks of age, and broiler breeder hens were fed a control diet or this diet supplemented with 0.25% Azomite from 21 weeks of age through 65 weeks of age. Heat stressed laying hens were fed a layer diet supplemented with equal levels of selenium derived from either sodium selenite (inorganic source), Sel-Plex® (organic source) or Selisseo® (organic source) from 42 through 71 weeks of age. In laying hens total marketable eggs, and in broiler breeder hens total settable eggs were increased ($P < 0.05$) with the dietary inclusion of Azomite by 8 and 9 eggs per hen, respectively. Marketable egg production was significantly ($P < 0.05$) greater (5 eggs per bird) for the hens fed Selisseo® relative to those fed sodium selenite, while marketable egg production in hens fed Sel-Plex® did not differ from the other 2 treatments. The results indicate that providing rare earth elements may be beneficial to egg production in hens and that feeding Selisseo® can lessen the detrimental effects of heat stress on laying hens.

INDEX WORDS: Aluminosilicate, rare earth elements, tibia ash, molt, C and P apparent digestibility, organic selenium, inorganic selenium, egg quality

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW OF AZOMITE

Trace Mineral Elements

Modern commercial layers and broiler breeders are bred for high reproductive efficiency, which equates to a need for optimal nutrient absorption in order to satisfy the dietary requirements for prolific production and maximal hatchability, respectively. One of the prominent obstacles with respect to egg production is the decrease in productive performance that is observed as laying hens age (Berry, 2003). Washburn (1982) specifically attributed this incidence to the observation that as eggshell size increases in aging layers, eggshell weight does not proportionately increase; thus, reductions in eggshell quality are inevitably seen. While studies in the early 2000s noted that shell defects can account for up to 8% of all eggs that are lost in production, recent reports have shown that nearly 10-15% of all eggs produced are lost prior to or during collection on account of eggshell defects (Klecher et al., 2002; Stefanello et al., 2014). Thus, it comes as no surprise that such incidences will impact the profitability of egg production and inhibit the economic status of the poultry industry.

Recent studies have particularly focused upon ameliorating these issues through the utilization of inorganic or organic trace mineral supplementation. Although the dietary requirements and inclusion levels of trace minerals are quite miniscule relative to other feedstuffs – incorporated into diets as milligrams per kilogram of the complete feed mixture – their biological significance is profound (NRC, 1994). While nutrients may modulate the immune system and thereby defend against pathogens by a multitude of means, micronutrient deficiencies are notably more debilitating to the maturation of the immune system than deficiencies of dietary energy and protein (Klasing, 1998; Dalgaard et al., 2018). Indeed, trace elements play pivotal roles as key components of proteins involved in immune function and intermediary metabolism while also serving as cofactors for a variety of metalloenzymes, including the selenium-dependent antioxidant enzyme glutathione peroxidase (Wang et al., 2019; Echeyerry et al., 2016). Therefore, trace minerals are essential to the functional activities of the organic moieties that require them (NRC, 1994). As discussed by Fernandes et al. (2008), trace minerals are particularly important with respect to enzymes involved in membrane and eggshell synthesis; in addition, trace minerals have also been shown to directly interact with the calcite crystals during eggshell mineralization (Nys et al., 1999). Thus, their physiological levels in the bird impact eggshell quality. In correlation with these findings, a study by Stanley et al. (2012) showed that supplementation with organic trace minerals selenium (Se) and zinc (Zn) effectively enhanced egg production, egg size and eggshell weight in post-molted laying hens. It is relevant to note that as laying hens age, the production of free radicals concomitantly increases; thus, the antioxidant protection requirements of older hens is relatively high (Stanley et al., 2012). Since establishing an effective antioxidant system is reliant upon the Se status of an organism, trace element supplementation is particularly imperative with respect to the productive lifespan of older laying hens (Stanley et al., 2012). Within the last few years, more studies have emerged that support the role that trace minerals play in the production and maintenance of a highly functional immune system. In light of evidence that zinc, copper, manganese, and selenium may serve as external antioxidants in the management of oxidative stress, Echeyerry et al. (2016) compared the effects of Monensin, bacitracin methylene disalicylate (BMD), and a BMD-diet supplemented with organic trace minerals on broiler performance (Willcox et al., 2004). The authors found that, relative to the other treatment groups, broilers supplemented with organic trace minerals showed higher villi height/crypt depth ratio, lower plasma malondialdehyde (MDA) content, and the up-regulation of IL-10 in the spleen (Echeyerry et al., 2016). These findings provide evidence that dietary supplementation with trace mineral elements may improve the immune status, intestinal development, and performance of broilers by combating oxidative stress and enhancing histomorphological aspects of the digestive system; therefore, further investigations into the impact of trace element supplementation on poultry performance are crucial as we move toward creating more defined and uniform diets.

Rare Earth Elements

Although the utilization of rare earth elements (REE) in Asian production systems has been commonplace since it became an area of focus in agricultural research in 1972, the interest in REE use in Western agricultural practices has taken longer to follow suit (Hu et al., 2004). The term REE applies to 17 elements that actually represent the 15th most abundant component (about 0.016%) of the earth's crust, whose individual content varies depending on the geographical region and soil type: cerium (Ce), lanthanum (La), europium (Eu), gadolinium (Gd), holmium (Ho), erbium (Er). thulium (Tm), ytterbium (Yb), lutetium (Lu), promethium (Pm), neodymium (Nd), praseodymium (Pr), samarium (Sm), terbium (Tb), yttrium (Y), and scandium (Sc) (Weber and Reisman, 2012; Hu et al., 2006). REE are classified into either one of two groups based on atomic weight: light rare earth elements, also recognized in order of atomic number as the cerium subgroup from lanthanum (57) to samarium (62), and heavy rare earth elements, also known as the yttrium subgroup from europium (63) to lutetium (71) (Zhuang et al., 2017). Even though scandium (Sc) and yttrium (Y) do not belong to the lanthanide series, which are the 15 elements ranging in atomic number from 57 to 71, they are considered to be

REE since they possess similar chemical properties (Zhuang et al., 2017). Establishing a greater understanding of the distribution of these elements is challenging due to the lack of sensitive tests as well as the abundance of diversity from sample to sample. What is evident is that China's soils appear to be one of the world's wealthiest sources of REE with an average of 176.67 mg/kg of based on 279 samples tested out of 400 samples that were collected globally; however, based on 30 testing samples, the USA had an average of 57 mg/kg of soil (Hu et al., 2006). Light REEs comprise over 90% of the REE content in China's soils, whereby lanthanum and cerium are the most dominant contributors (Hu et al., 2006).

While rare earth elements have been utilized in the production of food animals such as poultry as well as in aquaculture, there exists an array of conflicting data regarding the impact that REE supplementation, along with its inclusion levels, has on the performance parameters of each respective species. A recent study by Zhou et al. (2016) reported that increasing the inclusion level of REE in the diets of Gibel carp (*Carassius gibelio*) did not translate to improvements in performance. Interestingly enough, the authors found that Gibel carp fed diets supplemented with 0.08% (0.8 g/kg) REE gained more weight than those that were fed the control diet or this diet supplemented with 0.4 (4 g/kg) and 0.8% (8 g/kg) REE (Zhou et al., 2016). As reviewed by Lei and Xueying (1997), a variety of experiments that evaluated the impact of dietary REE-containing products on domestic fowl reported positive trends in performance. In a year-long study conducted by Zhang et al. (1989), standard laying hen diets were either supplemented with rare earth (RE) inorganic compounds containing 45% RE oxides or RE organic compounds consisting largely of organic salts and 30% RE oxides. The authors observed significantly increased egg performance in 'Binbai' commercial layers that received the dietary treatment supplemented with RE organic compounds at 30 mg/kg of the diet; indeed, these hens exhibited a hen/day egg production of 218.7 compared to 213.1 for the control birds (Zhang et al., 1989). While differences in egg mass were not observed in the previous study, Guo et al. (1993) found that laying hens fed diets supplemented with 10 mg/kg RE citrate from 75 weeks of age showed statistically significant improvements in daily egg mass. Although differences in eggs per 100 hen days were not reported as statistically significant, the laying hens that received the diet supplemented with 10 mg/kg RE citrate provided 62.1 eggs per 100 hen days while control birds provided 58.4 eggs per 100 hen days (Guo et al., 1993). In addition to differences in egg production, other studies have provided evidence that REE enhance feed efficiency; indeed, Wang et al. (1991) described feed conversion efficiencies of 2.46 and 2.39 in layers fed 300 and 500 mg/kg of RE, respectively. In addition to these findings, a study by Cai et al. (2015) assessed the efficacy of an REE/Yeast product on the performance of laying hens at 52 weeks of age and subsequently found egg production was improved after an experimental period of 4 weeks; however, it is important to note that the influence of the yeast may have impacted the results beyond that of the REE inclusion. Nonetheless, throughout literature, increased egg production and feed efficiency are commonly observed in laying hens supplemented with dietary inclusions ranging from 0.003 to 1% REE. With respect to breeders, Wang et al. (1991) found that upon feeding diets containing 0, 300, and 500 mg/kg RE nitrate for 30 weeks to Dekalb XL-Link breeding stock, hatchability was greater in both RE nitrate-supplemented groups compared to controls and fertility was significantly increased in breeders fed the diets supplemented with 500 mg/kg RE nitrate.

Statistically significant improvements in broiler performance have also been observed throughout the literature for REE supplementation. Wang (1989) reported significant improvements in carcass quality and meat composition upon supplementing broiler diets with

300 mg/kg and 500 mg/kg while Yang et al. (1990) found that RE oxide inclusion levels of 195 mg/kg in Hybro broilers increased eviscerated yield by 6-7%. According to a study by Guo et al. (1991), Lohmann broilers that received diets containing 20 mg/kg to 40 mg/kg RE nitrates showed improvement in body weight gain as well as feed conversion rates compared to controls and treatment groups that received diets either supplemented with 10 mg/kg or 80 mg/kg RE nitrate. These broiler results agree with the research of He et al., (2009) who reported improvements in body weight gain of Ross broilers that were fed REE supplements from day of hatch to 35 days of age in two separate experiments. The authors measured multiple blood serum parameters, including aspartate aminotransferase (AST), creatine kinase (CK), glucose, total protein, albumin, globulin, phosphorus, calcium, potassium, and sodium. Despite the wealth of parameters tested, the authors reported no differences (He et al., 2009). However, improvements in body weight gain and feed conversion have also been observed in Japanese quail (Coturnix coturnix) fed diets supplemented with REE from 0 to 4 weeks of age (Eleraky and Rambeck, 2011). Although these studies show promising results for the promotion of poultry productivity, inconsistent data remains a relevant hurdle with respect to REE studies. Indeed, in a series of studies carried out by Schuller et al. (2002), REE were added to diets in order to quantify performance alterations in swine and poultry production systems. The authors included a REE salt into their basal diet at 0.0075% and 0.03% inclusion rate and found that although higher daily weight gains and feed conversions were reported in swine, there were no significant changes in the performance of laying hens fed diets supplemented with REE.

Among the REEs, cerium (Ce) and lanthanum (La) have been the subject of many investigations and serve as the most commonly used REE in feed additives for poultry and swine (Pagano et al., 2015). Experiments with individual elements in REE deposits provide greater insight into potential mechanisms by which these major components of REE products are acting. Bolukbasi et al. (2016) evaluated the effect of cerium oxide supplementation at inclusion levels from 100 mg/kg to 400 mg/kg of the diet in Lohman Brown laying hens from 22 to 32 weeks of age in an effort to elucidate whether cerium oxide served as one of the individual REE that contributed to alterations in bird performance. Indeed, the authors confirmed this supposition upon observing that hens supplemented with cerium oxide showed improved feed conversion rates, increased egg production, and prolonged egg shelf-life compared to controls (Bolukbasi et al., 2016). In agreement with these findings, the shell breaking strength was greater for eggs laid by hens fed diets supplemented with 300 mg/kg and 400 mg/kg cerium oxide; however, albumen weight, yolk weight, shell weight, shell thickness, and specific gravities were the same across dietary treatments (Bolukbasi et al., 2016). Relative to the proposed antioxidant effects of REE, Bolukbasi et al. (2016) found that hens that received diets supplemented with 200 mg/kg, 300 mg/kg, and 400 mg/kg cerium oxide all had lower serum malondialdehyde (MDA) content and superoxide dismutase (SOD) activity than hens fed the control and 100 mg/kg inclusion level diets. These experiments suggest that cerium may be used in poultry production without negatively impacting performance; in fact, it may potentially reduce oxidative stress and thereby improve production. In a previous study, these authors added lanthanum oxide to the diets of laying hens at the same inclusion rates as those utilized in the cerium oxide-supplementation study. Durmus and Bolukbasi et al. (2015) reported that the greatest dietary inclusion rate (400 mg/kg) resulted in significantly greater egg production and lower feed conversion rates over the 10-week experimental period compared to the other dietary treatment groups. While serum Ca and P concentrations were unchanged across treatments, serum MDA content was reduced in hens fed 300 mg/kg lanthanum oxide (Durmus and Bolukbasi et al., 2015). In addition to these observations, the authors also reported lower MDA content in the yolks of eggs produced by hens in the 200 mg/kg, 300 mg/kg, and 400 mg/kg lanthanum oxide treatment groups.

Although the aforementioned results appear promising, dietary lanthanum supplementation in broilers has resulted in inconsistent bird performance results. While Agbede et al. (2011) reported that lanthanum oxide at 85.3, 171, and 256 ppm in the diet increased the total weight gain in Arbor Acres broilers in a 56-day experiment, a similar study conducted the next year did not detect performance differences between broilers fed the control diets or those fed diets containing 100, 200, 300, and 400 mg/kg of lanthanum oxide and lanthanum chloride (Igbasan and Adebayo, 2012). Authors from neither of the previously mentioned studies reported any significant differences in organ weights, carcass yields, biological nor haemotological parameters between the different dietary treatment groups. These observations indicate that lanthanum may not consistently alter broiler performance; however, it is important to note that the sample size for these studies (30 birds per treatment and 20 birds per treatment, respectively) is quite small, which leaves the opportunity open for larger-scale investigations that may provide further insight. In addition to this potential discrepancy between cerium oxides and lanthanum oxides, results of REE supplementation in production animals is not uniform; in fact, some data points to adverse effects on animal performance upon exposure to REE, which may be a consequence of over-supplementation (Pagano et al., 2015). According to Pagano et al. (2015), observations that REE follow hormetic concentration-related trends, whereby low inclusion levels promote stimulatory or protective effects while higher concentrations induce adverse effects, may explain the discrepancies seen throughout literature. However, the profusion of data on REE that has surfaced in the last few years has also been significant with respect to a rudimentary phenomenon in both animals and humans: geophagia (Panichev, 2015). The consumption of substances that are largely non-nutritive, but comprise compounds such as clays and constituents such as REE, have been proposed to be a means by which animals maintain ratios of rare earth elements and other mineral elements that either directly or indirectly impact the biochemical processes of the body that define health status (Panichev, 2015).

Aluminosilicates

Although the phenomenon of geophagia is rather primitive, there is a great amount of speculation among researchers about the driving forces of this behavior, particularly with respect to vertebrates. For the last few decades, scientific investigations have suggested that either the sodium or magnesium content of a given clay entices various species to exhibit geophagia (Emmons and Stark, 1979; Powell et al., 2009). These studies showed that the Peruvian deposits which attracted the attention of several species of animals, including parrots, were similar in clay content to other nearby clay banks; however, the sodium and magnesium content of the soils varied drastically. On the other hand, Gilardi et al. (1999) did not detect any differences between the natural clay licks and other local sources of clay in the Amazon Basin where animals were exhibiting pica; thus, these authors suspect that vertebrates carried out this behavior in an attempt to utilize the clays to bind dietary toxins and promote intestinal integrity. Although a consensus has yet to be reached, both arguments provide insight into the importance of clays with respect to animal nutrition.

Aluminosilicates regularly contain between 40% and 70% Al_2O_3 (aluminum oxide) and 10% to 20% SiO_2 (silicon dioxide) (Gilani, 2016). Aluminosilicate-based clays, including bentonite, zeolite, and kaolin, are included in diets to promote performance in food animal production systems. It is proposed that these clay products work via a variety of mechanisms, including slowing down gastrointestinal transit time and influencing the intestinal morphology and immunity (Quisenberry, 1968; Wawrzyniak et al., 2017; Jarosz et al., 2017; Wlazlo et al., 2016). In light of the evidence presented above regarding observations of geophagia across a multitude of animal species, scientists have utilized clay products in diets with varying degrees of success in their attempts to mimic nature. A particularly promising area of research with respect to aluminosilicates is toxin binding. Kubena et al. (1998) investigated a hydrated aluminosilicate product and its ability to mitigate the effects of mycotoxicosis in broiler chicks. Chicks were reared to 21 days of age with 5 mg/kg of aflatoxin added to the diet. In this study, the researchers reported that when challenged with aflatoxin, 0.250% and 0.350% inclusions of hydrated aluminosilicate in the diet significantly reduced the feed conversion rate as well as increased chick growth. Although these findings are favorable, the authors also reported that tricocethene (T-2) toxicity was not assuaged by the product, thereby indicating that not all toxins were bound by the hydrated aluminosilicate (Kubena et al., 1998). In support of these findings, a later study by Chen et al. (2014) inoculated broiler chicks with 0.5 to 2 mg/kg of aflatoxin B1 and administered diets supplemented with a hydrated sodium calcium aluminosilicate (HSCAS) product that was incorporated at 0.5%. By week 3 of the experiment, the authors found that the HSCAS product helped recover performance in inoculated groups (Chen et al., 2014). They also reported that the inclusion of HSCAS reduced the negative effects of the aflatoxin in the liver and increased expression of hepatic catalase and superoxide dismutase. Taken together, both studies provide strong evidence for the use of aluminosilicate products in mitigating the costly influence of dietary aflatoxins.

In addition to binding toxins in the diet, clays have been used to manage litter quality by decreasing NH_3 emissions (Luna et al., 2015; Wlazlo et al., 2016). Indeed, ammonia gas in excess of 25 ppm is considered harmful for a broiler’s health and thereby negatively impacts bird performance. Decreasing the amount of aerosolized ammonia decreases secondary insults to the respiratory tract, thereby promoting poultry performance. Wlazlo et al., (2016) investigated bentonite and zeolite in their ability to reduce ammonia. They reported that doses of 1% to 2% of either zeolite or bentonite added to laying hen manure reduced ammonia by almost 30% compared to the control, thereby providing evidence that the addition of aluminosilicate clays as a litter amendment is beneficial in the reduction of ammonia production (Wlazlo et al., 2016). Prasai et al., (2017) also reported linear decreases in environmental ammonia as the inclusion of biochar, bentonite, or zeolite (each at 1%, 2%, and 4% inclusions) increased in broiler diets over a 46-day period. In addition to these observations, broilers in all treatment groups performed better than the untreated control broilers in terms of body weight gain and feed conversion; however, these improvements were not proportional to the linear improvements observed in ammonia reduction. Although scavenging ammonia is imperative to the health of the broiler flock, this might not be the driving, let alone the only, factor explaining the improvement in broiler growth and feed utilization when aluminosilicate-based products are added to broiler diets. In the late 1960s, a series of experiments were conducted by Quisenberry (1968) in an effort to observe the effects of clay products on laying hen performance. Increased hen weights, increased egg weights, decreased mortality, and increased hen-day production in diets supplemented with bentonite at 2.5% and 5% inclusion levels were observed (Quisenberry, 1968). The control diet had an energy level of 945 kcal/pound, whereas the bentonite inclusion resulted in decreased dietary energy levels of 932 and 918 kcal/pound for the 2.5% and 5% diets, respectively. Though these diets had lower energy, the productivity of the hens remained similar – arguably better - than the control. From these observations, the author concluded that adding clay to the diet resulted in caloric-sparing effects; indeed, it was postulated that the increased feed utilization seen in aluminosilicate-supplemented hens was the result of a slower passage rate, which allowed for greater nutrient absorption (Quisenberry, 1968).

More recent publications have expanded upon the potential mechanisms by which aluminosilicates act. Indeed, in addition to binding toxins and potentially slowing the intestinal passage rate of digesta, observations of morphological and immunological changes with dietary inclusions of aluminosilicate products have become more prominent throughout literature. In a study with zeolite, an aluminosilicate of volcanic origin, Ross 308 broilers from 1 to 40 days of age were fed diets supplemented with 2% and 3% zeolite (Wawrzyniak et al., 2017). The authors reported that the zeolite increased villi surface area in the distal half of the small intestine (Wawrzyniak et al., 2017). As a complement to this, another study in the same year by Jarosz et al. (2017) reported that broilers fed diets supplemented with 2% and 3% zeolite had stimulated lymphocyte proliferation - specifically CD4 T cells - as well as increased pro-inflammatory cytokines such as interleukin 10 (IL-10) compared to broilers fed a control diet. Although the authors made a special note that dietary inclusions greater than or equal to 3% may cause intestinal inflammation and thus reduced performance parameters, both the 2% and 3% zeolite inclusions led to statistically increased body weights (Jarosz et al., 2017). In contrast to these studies, however, some researchers have reported no differences in poultry performance when different dietary inclusions of aluminosilicate products were evaluated. A year prior to the previously mentioned studies, Schneider et al. (2016) reported no significant differences in broilers fed 5 g/kg (0.5%) zeolites over a 42-day period compared to control diet-fed broilers. However, in this same study by Schneider et al. (2016), broilers raised on litter conditioned with 100 g/kg (10%) zeolite showed reduced litter moisture in this group compared to the control and the dietary zeolite inclusion groups, despite not having shown improved body weight gain or feed conversion rates. The inconsistencies observed with the use of aluminosilicate products in animal research may be expected given that parameters such as dietary toxin levels and ammonia production capacity are going to be highly variable from experiment to experiment. However, in light of the biological principles involved in toxin binding as well as the observations of decreased ammonia production and increased performance results in some investigations, aluminosilicate products can play a pivotal role in the enhancement of broiler production. Although there have been a variety of mechanisms proposed by the aforementioned research that defines how aluminosilicate products could positively affect broiler production, it is significant to address that unidentified nutrient(s) in some of these clay products may also contribute to promoting broiler growth by fulfilling unestablished nutrient requirements.

Azomite

Recent research developments have expanded the physiological and economic benefits of dietary inclusions of aluminosilicates, particularly with respect to hydrated sodium calcium aluminosilicates (HSCAS). AZOMITE® is a product marketed as a hydrated sodium calcium aluminosilicate that is comprised of trace minerals and rare earth elements. Mined near Nephi, Utah, which is south of Salt Lake City, Utah, “AZOMITE” is an acronym which stands for “A to Z of Minerals Including Trace Elements.” Hydrated sodium calcium aluminosilicates have been classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA). Similar to the aluminosilicates previously discussed, Azomite may be acting via multiple mechanisms including toxin binding, nutrient allowance, or the provision of some other benefit due to its distinct composition of hydrated sodium calcium aluminosilicate, rare earth elements, and other trace elements. Although Azomite contains a high proportion of aluminosilicate and is legally defined as such (HSCAS), its composition differs from other clays such as calcium bentonite clay due to the elements that compose them. While both calcium bentonite clay and Azomite are comprised of an aluminosilicate base, calcium bentonite clay only retains a total of 15 additional elements whereas Azomite regularly retains 74 elements. In fact, the concentration of REEs in Azomite is 529.7 mg REE/kg of soil (529.7 ppm total REEs), in which light REE comprise a total of 92.9% of the total REE content in Azomite (Azomite International, 2018). All elements that are considered REEs except for promethium (Pm), which is not typically found in the earth's crust, are retained in Azomite (Hu et al., 2006).

Geologists have described Azomite as a volcanic tuff breccia. Tuff and breccia are geological descriptors which both mean that a rock is composed of many different fragments and sediments that are bonded together with volcanic ash. A tuff is consolidated volcanic ash and dust that is also comprised of sediment (less than half). This ash sediment packs down to form the rock, and in doing so, forms small spaces within the rock. Breccia is similar yet is distinguished from tuff due to subtle differences. Breccia describes this compacted ash containing angular mineral fragments in excess of two millimeters within its compacted matrix (Ehlers and Blatt, 1982). Azomite is typically angular in appearance, though it can be rounded. Azomite does not fit perfectly into any of the three major categories in petrology: igneous, sedimentary, or metamorphic rock (Ehlers and Blatt, 1982). While its volcanic origin puts it squarely into the category of igneous rock, there also exists some influence from sedimentary rock. The rock forming the Azomite deposit is light pink or coral in color with black, gray, red, and yellow streaks and dots, which are various mineral deposits.

Although Azomite has been used in agriculture for over 70 years, there are a limited amount of scientific studies examining the use of the product in animal nutrition. Initially, the product was used as a soil amendment for plant growth, yielding results in numerous horticultural species. In particular, peer-reviewed university trials remain limited at this time; however, there are reports of Azomite being used successfully for the cultivation of peaches, citrus, figs, tomatoes, wheat, and grapes (Azomite International, Studies and Tests). In recent years, research with Azomite has gravitated away from its use as a soil amendment to its potential use in agricultural animal production systems. With respect to feed milling, Azomite has been utilized for its anticaking properties. Tillman et al. (2020) recently reported that the inclusion of Azomite at 0.25% and 0.50% significantly improved pellet production rates when meat and bone meal and/or dried distiller's grains with solubles (DDGS) were used in the diet at 4% and 8%, respectively. Evidence that Azomite enhances feed mill throughput while maintaining pellet quality is advantageous as it can be useful in offsetting some of the negative effects that DDGS imposes on pelleting production rate (Tillman et al., 2020). Indeed, the hope is that Azomite's ability to mitigate such effects will inevitably translate to decreases in pellet mill energy and thus solidify Azomite's potential as an alternative to the inorganic phosphates that are traditionally used to increase pellet throughput (Tillman et al., 2020).

Other aspects of agricultural production, such as investigations in aquatic species, have suggested that Azomite may enhance the immune systems of organisms such as shrimp and tilapia, thereby promoting growth (Liu et al., 2011; Tan et al., 2014). Research with Azomite has also been prominent in carp species, which are the most common fish produced in aquaculture worldwide. Liu et al. (2011) conducted a study in grass carp (Ctenopharyngodon idella) in which diets were supplemented with 0.2% Azomite for an 8-week experimental period in order to assess its impact on performance. The authors found that carp fed a diet supplemented with 0.2% Azomite showed increased growth and lower feed conversion rates than carp fed a control diet void of Azomite; however, feed conversion rates were not altered when dietary inclusion rates of 0.3% and 0.4% Azomite were supplied (Liu et al., 2011). Of notable interest is that Liu et al. (2011) reported increased intestinal protease, lipase, and amylase levels as well as higher superoxide dismutase levels in the serum of carp fed diets supplemented with Azomite, irrespective of inclusion level. From these observations, the authors concluded that Azomite was positively modulating the non-specific innate immune system in addition to increasing intestinal enzyme activities (Liu et al., 2011). Due to their observations with respect to growth and feed conversation rates, along with the discovery that intestinal enzyme activity and the non-specific innate immune system were upregulated regardless of dietary inclusion level, the authors suggested that supplementation with 0.2% Azomite is likely the most ideal inclusion rate (Liu et al., 2015). In the same year, Jaleel et al. (2015) conducted a comparable 8-week investigation in koi (Cyprinus carpio) fingerlings, an ornamental carp species, in which similar inclusion rates of 0.0%, 0.2%, 0.4%, and 0.6% Azomite were utilized in the diets. Findings differed a bit from those observed by Liu et al. (2011). In contrast, Jaleel et al. (2015) found that growth rates and immune parameters were the most improved in carp fed diets supplemented with 0.4% Azomite.

Tilapia (Oreochromis sp) are second to carp in terms of worldwide aquaculture finfish production. Prior to studies in carp, Liu et al. (2009) studied how the performance of tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) was altered by inclusion levels of Azomite at 0%, 0.25%, 0.50%, and 0.75% of the diet over a 30-day experimental period. Each dietary treatment consisted of 3 replicates of 20 fish, and intestinal morphology as well as some serum parameters such as superoxide dismutase were also measured (Liu et al., 2009). The authors found that all dietary treatments containing Azomite had increased weight gain and decreased feed conversion rates. Relative to the control group, tilapia fed diets containing 0.25% and 0.50% inclusion levels of Azomite showed significantly increased villi height and width. Tilapia from these two dietary treatments also had increased protease activity in their intestine and stomach, increased dry matter digestibility, and increased Lactobacillus numbers in the intestine (Liu et al., 2009). In addition to these findings, Liu et al. (2009) also found that tilapia fed diets containing 0.25% Azomite had higher level of superoxide dismutase and lysozyme than controls. A few years later, Azam et al. (2016) replicated this investigation by Liu et al. (2009) in male tilapia (Oreochromis sp.) over a 49- day experimental period. Azomite inclusion rates in the diets ranged between 0%, 0.25%, 0.5%, and 0.75%. Azam et al. (2016) found that tilapia fed the higher inclusion rates of Azomite – 0.5% and 0.75% - had greater body weight gain and lower feed conversion rates than controls; however, no variance in lipase enzymes were observed across the dietary treatments.

To look further into the functionality of the immunomodulatory alterations in tilapia, Musthafa et al. (2015) designed a challenge study with Aeromonas hydrophila. This particular bacterium is known to increase mortality in aquatic species, including fish and amphibians. The authors included a positive and negative (infected) control as well as three dietary treatment groups that were comprised of varying inclusion levels of Azomite (2 g/kg, 4 g/kg, and 6 g/kg). Each treatment group was represented by 3 replicates of 25 tilapia. Thirty days following acclimation to the dietary treatments, the tilapia were inoculated with 100 microliters of PBS with 3.1 x 107 cfu ml-1 of Aeromonas hydrophila in the peritoneal cavity. Blood was collected on weeks 1, 2, and 4 post-inoculation. On weeks 2 and 4 post-inoculation, the tilapia fed 4 and 6 g/kg inclusion levels of Azomite showed increased lysozyme activity, respiratory burst (measured via reactive oxygen species), and lower mortality compared to the other dietary treatment groups.

Investigations in Azomite-supplemented shrimp have had similar results to those of the finfish studies. Tan et al. (2014) reported that Pacific white shrimp (*Litopenaeus vannamei*) fed diets supplemented with 2.0 and 4.0 mg/kg of Azomite relative to control diet-fed shrimp showed statistically significant improvements in weight gain and feed conversion ratios over the 6-week experimental period. The authors also exposed the shrimp to artificially-induced hypoxic conditions in order to induce stress and found that resistance to stressors and survivability upon inoculation with Vibrio alginolyticus were both improved in shrimp fed diets supplemented with Azomite. Significant increases in stomach protease, hepatopancreas lipase, serum lysozyme, and phenoloxidase were observed in the shrimp fed diets supplemented with 4.0 g/kg Azomite. While shrimp fed the lower inclusion level (2.0 g/kg) of Azomite exhibited similar trends in enzyme levels, these shrimp also had significantly increased levels of superoxide dismutase. Interestingly, shrimp fed diets with higher inclusion rates of Azomite (6.0 and 8.0 g/kg) did not exhibit the improvements in performance seen with the lower inclusion rates; however, they exhibited increased serum lysozyme and phenoloxidase levels compared to controls. The enhancements in body weight gain and feed conversion rates noted in the Azomite-treated groups (2.0 mg/kg and 4.0 mg/kg) may best be explained by the increase in digestive enzymes observed in these dietary treatment groups.

With respect to poultry production, Azomite has also been reported to promote positive performance attributes in broilers. In an early meta-analysis by Emerson and Hooge (2008), 13 contract research farms and 10 integrator trials reported increases in breast meat yield by 0.70% and 0.38%, respectively. In these investigations, dietary treatment groups contained Azomite in the diet while control groups received a standard diet without the inclusion of Azomite; however, the abstract from this 2008 meeting does not include details regarding the dietary inclusion rates of Azomite, the number of replicates in the study, nor details concerning the environmental conditions and experimental design. A decade later, Malheiros et al. (2018) conducted a trial in W36 Hy-Line laying hens from 67 to 85 weeks of age in which hens either received a standard laying diet or the same diet supplemented with 0.25% Azomite. The experimental design included a non-fasting molting period and consisted of 96 W36 Hy-Line laying hens (48 laying hens per dietary treatment group). Body weight, feed intake, feed conversion ratio, and egg quality parameters (shell color, egg weight, Haugh unit, yolk color, and shell thickness) did not differ between the treatment groups. However, at 85-weeks of age, during the post-molt period of the experiment, the hens receiving 0.25% inclusion levels of Azomite had improved percent hen housed egg production compared to the controls. Since there was no mortality, the percent hen day egg production was identical to the percent hen housed. In addition to these observations, the hens fed Azomite also had lighter tibia bone weight compared to controls. The authors attributed the lighter tibia bones to the consequence of increased egg production; however, further investigations into the driving forces behind the enhanced egg production are still necessary. In light of the immunomodulatory effects that Azomite has been shown to have in the realm of aquaculture, it would be most advantageous to evaluate similar parameters in poultry such as plasma superoxide dismutase, as its activities are heavily intertwined with the stress response and may provide further insight into the biological mechanics of Azomite (Fournier et al., 2000).

Although there is not a wealth of research surrounding the potential benefits of Azomite with respect to the poultry industry, investigations into its use as a feed additive in broiler production have increased over the last year. According to a study by Pirzado et. al (2020) that was conducted earlier this year, the influence of Azomite in conjunction with a low-energy diet on the growth performance, bone parameters, and nutrient digestibility of broilers provided some positive results. One day-old Arbor Acres broiler chicks were randomly assigned into 1 of 3 dietary treatment groups: Controls fed a basal diet, LME (Low metabolizable energy) fed a basal diet – 100 kcal ME kg-1, and LME fed a diet supplemented with 0.25% Azomite (AZO-0.25%). Each dietary treatment group consisted of 6 replicates and 10 birds per replicate. Unfortunately, the authors did not specify the length of the experimental period nor any additional details regarding the environmental conditions. At the end of the study, however, the authors found that live body weight and average daily gain were significantly improved while feed conversion ratio was lower in broilers fed LME diets supplemented with 0.25% Azomite compared to broilers fed the LME diets. In addition to these observations, broilers fed the AZO-0.25% diet had greater dry matter, crude protein, apparent metabolizable energy (AME), phosphorus (P), and calcium (Ca) digestibility relative to broilers fed the LME diet. The authors additionally evaluated bone parameters; indeed, Pirzado et al. (2020) found that tibia length, tibia breaking strength, ash, and P and Ca percentages were significantly greater in the AZO-0.25% birds relative to broilers fed the LME diet, which provides some evidence that Azomite may potentially serve as a means of reducing energy in the diet while promoting growth performance and nutrient digestibility. However, statistical analyses comparing the AZO-0.25% birds to those that received the control diet were not discussed in the abstract; thus, the preceding notions are drawn without a full comprehension of the results and any factors of environmental design that may have influenced them. Preceding the aforementioned study, a larger scale evaluation of Azomite use in broilers was conducted in a 32-day study by Ahamed et al. (2019), in which 1,020 day-old Cobb 500 male broiler chicks were randomly assigned to 5 dietary treatment groups with 6 replicates per treatment (34 chicks/pen). The dietary treatment groups were as follows: (1) Controls fed a basal diet without Azomite, (2) basal diet with 0.25% Azomite, (3), basal diet with 0.5% Azomite (manufacturer's recommendations), (4) basal diet with 0.75% Azomite, and (5) basal diet with 1.0% Azomite. By the cessation of the study, Ahamed et al. (2019) found that, overall, supplementation with Azomite had a statistically significant effect on the growth performance of broilers. Although the authors state that live body weight (LBW), body weight gain (BWG), average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR), and gain cost of the birds fed various levels of AZOMITE® were significantly improved as compared to the control, the statistical breakdown of the data was not provided. However, the authors suggested that supplementation of AZOMITE® in commercial broiler at 0.50% to 1.0% improved live weight, feed intake and feed conversion ratio of commercial broilers and are therefore the ideal dietary inclusion levels of Azomite. As noted with the previous studies involving the utilization of Azomite, the literature is vague in its details of experimental design and results; thus, the scarcity of literature with respect to Azomite use in poultry production remains as a driving force for more thorough investigations in the future.

Summary

Although the dietary inclusion of Azomite may provide the performance benefits seen previously with the individual addition of aluminosilicate clays or REE's in poultry production, there lacks a wealth of research exploring the utilization of Azomite in poultry. One of the most notable features of Azomite is its aluminosilicate base, which could be pivotal to the mechanisms by which Azomite potentially acts due to the ability of aluminosilicates to bind toxins, improve feed efficiency, and promote immunological function across a variety of production systems. Further research is necessary in order to determine Azomite's capacity to promote poultry performance as well as elucidate the mechanisms by which it may modulate the biological efficiency of the bird.

CHAPTER 2

LITERATURE REVIEW OF SELENIUM IN POULTRY PRODUCTION

Selenium in Feed Formulation

Originally recognized in animal diets for its toxicity, selenium (Se) has since been established as a trace mineral of profound importance. Upon the discovery by Schwartz and Foltzs (1957) that inorganic selenium salts effectively prevent necrotic liver degeneration, selenium has become the focus of many dietary additive investigations, particularly in poultry. Its biological significance cannot be overstated – beginning with its participation as a major constituent and determinant of selenoproteins as well as the turnover of selenoprotein mRNA levels, respectively (Gao et al., 2011). In fact, of the 26 genes encoding various selenocysteine-containing proteins that have been described in the chicken, a number of these selenoproteins have been identified as participating in redox balance in the cell (Lei, 2017; Surai et al., 2018). Such selenoproteins are therefore critical to antioxidant enzyme activities, immune function, reproductive performance, and general growth and maintenance (Ahmad et al., 2012). The devastating effects of Se deficiency in poultry have included general observations of decreased performance parameters; however, exudative diathesis, nutritional muscular dystrophy, encephalomalacia, and immunodeficiency have also culminated when Se inadequacy exists in cohort with vitamin deficiencies such as limited vitamin E (Bartholomew et al., 1998; Combs and Hady, 1991). Even though it is no longer common to see these issues in commercial poultry production, the optimization of growth rate and reproductive performance in modern birds have adversely impacted their sensitivity to variable stresses; therefore, it is not uncommon to observe reduced performance in broilers, broiler breeders, and laying hens alike when dietary levels of Se are not sufficient (Surai et al., 2018). Thus, it is unsurprising that optimal dietary inclusions of Se promote enhanced immune responses and improved growth and reproductive performance (Habibian et al., 2015). Although a multitude of Se deficiency syndromes are exacerbated by coexisting vitamin and mineral insufficiencies, Cantor et al. (1975) described the prevention of nutritional pancreatic atrophy (NPA) in chicks, which is particularly notable since its development is solely the result of a Se deficiency (Surai, 2002). Identifying ideal levels of dietary Se supplementation so as to provide a safe range between deficiency and toxicity is therefore paramount; however, the Se requirements of laying hens established by the 1994 NRC – 0.05 to 0.08 mg/kg of diet – have been challenged throughout recent literature. While the scope of recommended dietary intake of selenium by the NRC (1994) is quite narrow, Se toxicity in poultry has often only been noted when doses 10 times greater than the physiological requirement are administered (Surai, 2002). In fact, the maximal level established by the Association of American Feed Control Officials (2003) is 0.3 mg of Se/kg of the diet – a level which is frequently utilized to dictate the inclusion rate of premixes at levels of 0.2-0.3 mg/kg of complete feed without accounting for the Se content in other feed ingredients within the mixture (Słupczyńska et al., 2018; Surai and Fisinin, 2014).

While the physiological requirements of Se established by the 1994 NRC recommendations have been challenged by investigations that employ either large particle inorganic and organic sources, other studies such as those conducted by Zhou et al. (2011) showed that supplementation with Se nanoparticles up to 0.50 mg/kg of the diet improved daily body weight gain, feed conversion, and final body weights in broilers. In accordance with these observations, a later study by Qu et al. (2017) showed that supplementation of Se nanoparticles at 0.5 mg/kg of the diet in laying hens exposed to deoxynivalenol (DON) toxicity enhanced the rate of egg production, decreased the incidence of soft-shelled and cracked eggs, and additionally improved the total antioxidant status of the birds. These observations provide biological evidence that not only may explain the significant differences in production and essentiality of Se with respect to immunological status, but also provide testimony to the argument that greater inclusion levels of Se in the diet may be beneficial. In addition to the disagreement between the NRC (1994) recommendations and commonplace Se inclusion rates in complete feed mixtures, the Se content of feedstuffs also vary greatly depending upon the geographical region in which they were grown (Surai and Fisinin, 2014). Indeed, some investigations found that corn and rice grown in normal and high Se-containing soil may vary in Se content by up to 500-fold (Surai and Fisinin, 2014). Such differences would translate to variance in animal performance. This complication gave rise to the solution of creating uniformity by use of Se supplements. In fact, by early 1970, Se deficiencies in livestock became commonplace, and by 1974, the FDA approved the inorganic Se additives, selenite and selenate, for swine and poultry (Surai et al., 2018).

Sodium Selenite

Feed uniformity is particularly imperative when a narrow range between physiological requirement and toxicity exists - such is the case for sodium selenite (Vukacinovic et al., 2006). Although earlier investigations such as Cantor et al. (1975) indicated that organic dietary forms of Se more effectively protected broiler chicks against pancreatic degeneration than their inorganic counterparts, evidence that dietary form acts as a pivotal determinant of efficacy has mostly come to light over the last few decades (Surai and Fisinin, 2014). While sources of Se can be partitioned into either one of two categories – inorganic and organic – there exists a multitude of compounds and products that fall within those categories, such as sodium selenite or selenate and selenomethionine in the form of selenium-enriched yeast or SeMet hydroxy analogs, respectively (Surai and Fisinin, 2014). Although sodium selenite served as one of the original and most utilized forms of Se supplementation, its inclusion in feed formulations still persists today despite evidence that the natural form of Se, selenomethionine (SeMet), may be more advantageous with respect to levels of absorption and thus efficacy (Surai and Fisinin, 2014). Indeed, although it is more cost-effective to use inorganic sources of Se such as sodium selenite over organic sources such as Se-enriched yeast, there are a number of disadvantages associated with inorganic forms of Se including relatively high toxicity levels and adverse interactions with other minerals (Surai and Fisinin, 2014; Dalgaard et al., 2018). Vukacinovic et al. (2006) elaborated upon the issue of toxicity when they compared relative distributions of sodium selenite to Sel-Plex®, a source of organic Se in the form of selenized yeast, within feed mixtures. In their 2006 study, Vukacinovic et al. found that there was improved homogenization of the Se additive to the basal feed mixture when Sel-Plex® was used relative to sodium selenite. The authors attributed this observation to the difficulty of mixing a small amount of sodium selenite to a large basal diet. The statistical significance of the variance that was observed between levels of Se when different dietary forms were added to the basal diets, in combination with the relatively high toxicity of sodium selenite, provides evidence that there is a greater vulnerability to accidental Se overdose with respect to sodium selenite use (Vukasinovic et al., 2006).

On the opposite end of the spectrum, due to its inability to compile and maintain Se reserves as well as its negative interactions with other compounds, a large proportion of sodium selenite that is consumed is inevitably excreted (Surai, 2002). In a study carried out by Bai et al.

(2017), inorganic trace minerals were supplied in poultry diets at both 2 and 10-times the NRC recommended levels (NRC, 1994) in an effort to ensure that deficiencies would not arise due to the presence of antagonists within the diet. This creates a matter of contention, however, because supplementing diets with greater levels of inorganic trace minerals can then cause bioavailability interference with respect to other nutrients in the diet (Suttle, 2010). For instance, combining sodium selenite and ascorbic acid into a premix results in the reduction of selenite to metallic Se – a form of Se that cannot be absorbed (Surai and Fisinin, 2014). In this case, the biological activities of both nutrients are squandered because the chemical reaction between sodium selenite and ascorbic acid also results in the oxidation of ascorbic acid (Surai and Fisinin, 2014). While low retention rates and obstructive interplay with other vitamins and minerals must be taken into consideration when formulating diets with inorganic forms of Se, the efficacy of sodium selenite is also hindered by evidence that the selenite ion possesses oxidative stress-inducing effects (Spallholz, 1997). Indeed, in order to observe variations in reactive oxygen species generation, Terada et al. (1999) intravenously administered total parenteral nutrition fluids that contained either inorganic or organic forms of Se in the presence of sulfhydryl compounds. These studies showed that the addition of sodium selenite results in a greater loss of cellular protein as well as increased cellular damage compared to either selenate or selenomethionine (SeMet) under the same conditions (Terada et al., 1999). From these results, the authors concluded that selenite generates active oxygen species and thereby promotes cellular damage when exposed to sulfhydryl compounds – an observation that was not duplicated by either selenate or SeMet (Terada et al., 1999). In addition to these findings, Garberg et al. (1988) elaborated upon the role that selenite plays in generating DNA damage through its reaction with tissue thiols. Reactions between selenite and compounds such as glutathione produce selenotrisulfides, which then go on to react with other thiols, which then give rise to reactive oxygen species, namely superoxide radicals, via redox catalysis (Seko et al., 1989; Mezes and Balogh, 2009). According to a study carried out by Attia et al. (2010), poultry diets supplemented with sodium selenite at 0.3 ppm can damage the intestinal structure of chicks through the degeneration of the epithelial cells that line the intestinal crypts of the duodenum. In correlation with this, Attia et al. (2010) also found that the intestinal glands in the ileum of chickens fed 0.3 ppm sodium selenite suffered became necrotic. On the other hand, Yan and Spallholz (1993) discovered that SeMet does not engage in these mechanics of oxidative stress; however, in their studies, both sodium selenite and selenocysteine were shown to produce superoxide radicals and hydrogen peroxide in the presence of glutathione. It can thus be concluded that different selenium compounds possess different capacities to produce superoxide radicals (Mezes and Balogh, 2009). With respect to modes of potential toxicity, however, selenomethionine and selenocysteine have been found to be far less toxic than sodium selenite; in fact, it has been proposed that selenomethionine serves antioxidant functions rather than prooxidant (Spallholz, 1994; Surai, 2002). In contrast to this, sodium selenite possesses a low transfer efficiency of Se to the egg and developing embryo, which translates to little antioxidant defense against oxidative stress that coincides with hatch (Surai, 2006; Surai et al., 2016).

Selenium-enriched Yeast

Selenium-enriched yeasts provide another avenue of dietary selenium supplementation. As described by Combs and Combs (1984), the chemical and physical properties of selenium and sulfur are quite similar, and therein lies the opportunity to utilize plants' inability to differentiate between them with respect to amino acid synthesis and thus generate SeMet when selenium becomes available (Surai and Fisinin, 2014). However, the mass production of Se-enriched yeast is far more commercially convenient than the use of Se-accumulating plants (Kitajima and Chiba, 2013). Indeed, while forage crops convert a majority of Se into SeMet, yeast strains such as *Saccharomyces cerevisiae* are capable of assimilating up to 3,000 mg of Se/g and incorporate the primary product of this process, SeMet, into yeast proteins (Demirci, 1999; Rayman, 2004). According to the FDA, selenium-enriched yeasts derive from non-viable yeast, often *Saccharomyces cerevisiae*, that is cultivated in a fed-batch fermentation process whereby selenium salts, such as selenite or selenate, and cane molasses are allocated so that the adverse effects of selenium salts upon the growth rate of the yeast are curtailed (FDA). Culturing *Saccharomyces cerevisiae* with these inorganic Se compounds allows for the generation of organic Se compounds that possess greater bioavailability as well as considerably less toxic potential than sodium selenite and selenate in their original form (Nakamuro et al., 2000). While this process allows the inorganic selenium to be effectively incorporated, additional washing protocols are followed so that residual inorganic selenium is discarded and the final selenium yeast product does not contain greater than 2% of inorganic selenium in its total selenium content (FDA). In fact, the selenium in selenium-enriched yeast is often in the organic forms SeMet or SeCys (selenocysteine), whereby the proportion of SeMet can vary between 60-85% whereas the proportion of inorganic selenium ion is roughly less than 1% of the total Se (Slupczynska et al., 2018). According to Vukasinovic et al. (2006), there is additionally a better homogenization of Se, in the form of SeMet, in selenium-enriched yeasts compared to inorganic Se alternatives.

The type of yeast that is utilized ultimately determines its efficacy as a source of selenium (Ryszka et al., 2002). An early literature review by Rayman (2004) described the variation of SeMet percentages between different selenium-enriched yeast products, in which the

SeMet content of Se-yeast supplements such as SelenoExcell® varied between 60-70% of total Se while a commonly used Se-yeast product in poultry, Sel-Plex®, contained SeMet content anywhere from 62-74% of total Se. While such deviations in SeMet content can be attributed to differences in the production of the SeYeast, such as the strain as well as the source of Se that was used, the effectiveness of the extraction technique utilized by the different analytical laboratories also serves as a cofounding factor (Rayman, 2004; Surai and Fisinin, 2014). Surai and Fisinin (2014) also noted that there is great difficulty in releasing all of the SeMet in SeYeast preparations since a portion of that SeMet is bound up in resistant membrane hydrophobic proteins, thereby further contributing to observed differences in SeMet content. Between variations in fermentation conditions, energy sources (molasses), protocols of Se additions, pH, temperature, and incubation times utilized by different laboratories, it is inevitable that differences in SeMet proportions will occur (Esmaeili and Khosravi-Darani, 2014). Specifically, Mapelli et al. (2012) elaborated upon the significant impact that the level of sulfur supplied during the process of fermentation has on the upstake of Se as well as the Se-metabolite profile of yeasts. In addition to these variations, Simon et al. (2013) tested two different commercial SeYeast products in broilers that differed in SeMet content – 63% and 56.7% - and found that tissue selenium deposition between the two experimental groups varied. Since such differences between the two SeYeast preparations were directly proportional to SeMet content, it can be concluded that batch variability may be a critical drawback to the use of selenium-enriched yeasts (Simon et al., 2013; Surai and Fisinin, 2014). The lack of consistency in SeMet proportions in a singular product presents a critical issue to poultry nutritionist since SeMet proportions in SeYeast products are not provided on the label (Surai et al., 2018). Indeed, instead of SeMet proportions, product labels only show total Se as well as the inorganic proportion of Se as these are the properties that are officially regulated (Surai et al., 2018). Although Surai et al. (2018) proposed that it would be most conducive to balance Se proportions in the diet based off of SeMet instead of total Se, the author also acknowledged that it wouldn't be feasible to due to the aforementioned factors.

Aside from SeMet content serving as a determinant of tissue selenium deposition, studies such as those carried out by Juniper et al. (2011) found that when female turkeys were allotted graded additions of either SeYeasts or sodium selenite, proportions of total Se – as SeMet and SeCys – deposited into tissues significantly varied with tissue type as well as dietary treatment. While results showed that SeCys was the major form of Se in visceral tissues, Juniper et al. (2011) also found that muscle tissue glutathione peroxidase (GSH-Px) activity also mirrored the SeCys content that was present; however, regardless of these findings, differences in meat quality were not observed between dietary treatments. As will be discussed later, the variation in GSH-Px activity is particularly notable because GSH-Px is a pivotal selenoprotein in that it catalyzes the reduction of reactive oxygen species, thereby combating oxidative stress (Arthur, 2000). Due to the importance of selenoprotein synthesis, and its upregulation during times of stress, it is imperative that proportions of SeMet in SeYeast preparations are specified as this directly correlates with the Se reserves available for SeMet production. (Surai, 2006; Surai and Fisinin, 2014). Of course, one of the additional aspects to consider when weighing the advantages and disadvantages of SeYeast products is that cost-effectiveness would therefore depend on the level of SeMet in each product (Surai and Fisinin, 2014).

Selenomethionine (SeMet)

Pure SeMet is currently one of the leading supplementary options used to enhance the Se status of poultry (Schrauzer and Surai, 2009). Indeed, with respect to broiler breeders and their progeny, a variety of studies within the last decade have provided substantial evidence that SeMet is a superior source of Se compared to its inorganic counterparts. Wang et al. (2011) found that dietary maternal SetMet significantly increased selenium concentrations in the serum, kidney, liver, and breast muscle of broiler breeders while concomitantly increasing selenium deposition in the yolk, albumen, as well as the tissues of their 1-day-old progeny. In correlation with these findings, 1-day-old chicks of broiler breeders that received SeMet exhibited increased superoxide dismutase and glutathione peroxidase activity in the breast muscle, greater glutathione concentration in the kidney, decreased malondialdehyde concentrations in the liver and pancreas, and enhanced total antioxidant capacity (TAC) compared to chicks that derived from sodium selenite-supplemented broiler breeders (Wang et al., 2011). Thus, the antioxidant status of the 1-day-old chicks was notably improved by maternal SeMet intake (Wang et al., 2011). When Yuan et al. (2011) supplemented 48-week-old Lingnan Yellow broiler breeders with either sodium selenite, SeYeast, or SeMet for an 8-week-long experimental period, similar findings were presented – that is, the Se retention efficiency is maximized in 1-day-old chicks as well as in broiler breeders when broiler breeders are supplemented with SeMet compared to either SeYeast or sodium selenite. In contrast to Wang et al. (2011), however, Yuan et al. (2011) also included varying dietary levels of Se in their experimental diets and found that hatchability significantly decreased as dietary levels of Se increased across the different forms of Se. With respect to broilers, SeMet supplementation has also been shown to increase meat quality, which is not surprising considering that the seleno-amino acids SeMet and SeCys comprise roughly 91% of the total selenium content in chicken muscles (Bierla et al., 2008; Schrauzer and Surai, 2009).

Although there are conflicting results throughout the literature, numerous investigations have shown that the configurational stereochemistry of SeMet supplied to the diet may impact poultry performance. In comparison to sodium selenite, dl-SeMet has boosted GSH-Px and total antioxidant activity in the liver as well as enhanced the overall immunity in chickens (Bakhshalinejad et al., 2017; Wang et al., 2016). As reviewed by Surai et al. (2018), l-SeMet has been shown to have greater efficacy with respect to enhancing average daily gain, feed conversion ratios, and overall antioxidant defenses compared to d-SeMet. Upon evaluating the effect of either dl-SeMet or sodium selenite on the antioxidant status of broilers, Jiang et al. (2009) found that there were no significant differences between the two treatments with respect to GSH-Px activity and total antioxidant activity; however, superoxide dismutase (SOD) activity was significantly greater in the breast muscles of broilers that received dietary dl-SeMet. According to the EFSA, selenium deposition is not anticipated to deviate between the use of dl-SeMet and other SeMet sources (EFSA, 2014). However, further investigations will provide a better picture of how these configurational differences may vary in their influence upon the antioxidant status of poultry.

Selenomethionine Hydroxy Analogue: Selisseo®

In the last decade, pure molecules of organic selenium, known as selenomethionine hydroxy analogues (R,S-2-hydroxy-4-methylselenobutanoic acid; HMSeBA), have seen exponential increases in animal nutrition investigations. HMSeBA is the product of multiple reactions that inevitably give rise to a final product that contains selenomethionine hydroxy analogue content that is no less than 98% of the total Se content – and the total organic Se content of the additive itself is no less than 99% of total Se (FDA). A multitude of studies, including those by Briens et al. (2013) and Jlali et al. (2013), have provided evidence that supplementation with an HMSeBA product, called Selisseo®, results in greater tissue Se deposition in contrast to SeYeasts in broilers and laying hens, respectively. In fact, Jlali et al. (2013) found that hens supplemented with HMSeBA had significantly higher Se deposition in their eggs – by up to 28.8% - compared to those supplemented with SeYeast. As reviewed by Surai and Fisinin (2014), these findings could be particularly important to broiler breeders and newly hatched chicks vulnerable to oxidative stress. This is even more relevant as regulations such as those imposed by the EU have limited the maximum allowable Se content of SeYeasts to 0.2 mg of Se/kg of complete feed; indeed, under such conditions, the transfer efficiency of Se to the egg is even more critical (Surai and Fisinin, 2014).

With respect to broilers, Briens et al. (2013) ran two separate experiments that assessed the relative bioavailabilites of different inorganic and organic Se sources compared to Selisseo®. Bioavailability was evaluated by two parameters: muscle transfer efficiency and apparent digestibility (ADSe). In the first experiment, the authors found that Selisseo® increased muscle Se enrichment by an average of 1.48-fold compared to the selenium deposition in the muscle achieved by supplementation with the other Se sources (Briens et al., 2014). Upon evaluating the seleno-amino acid speciation for both Selisseo® and SeYeast, the authors found that muscle Se was only present as either SeMet or SeCys (Briens et al., 2013). These results provided sufficient evidence that since there was a full conversion of Se from the selenomethionine precursor by the birds, HMSeBA demonstrated both higher bioavailability for total Se as well as greater Se deposition efficacy due to increased muscle Se concentrations compared to SeYeast (Briens et al., 2013). To explain these observations, the authors turned to the differences in SeMet level in each of the dietary treatments. While the SeMet level in SeYeast varies from 60-70% - a consequence of batch-to-batch variability as discussed earlier – Selisseo® contains a nearly pure product of selenomethionine hydroxy analogue (purity > 99% HMSeBA), which may explain why the relative bioavailability for total Se increased by 39% in Selisseo®-supplemented birds compared to those supplemented with SeYeast (Briens et al., 2013). The authors also noted that Selisseo® increased the SeCys level in the muscle relative to the other Se sources, which could potentially support the idea that biochemical deviations in Se metabolism were the driving force since SeCys serves as the active form of Se via selenoproteins (Briens et al., 2013). Indeed, a previous study by Martin-Venegas et al. (2006) showed that the sulfur analogue of HMSeBA was more oriented towards the transsulfuration pathway than dl-methionine, which corroborates the argument that a specific pathway may be preferred when SeCys derives from a particular source or is provided in higher proportions, and therefore contributes to observed differences in bioavailability (Briens et al., 2013). With respect to SeCys levels, it is imperative to note that it is an indicator of active selenoproteins and is thus an indication of antioxidant status – a parameter that is critical to the performance of poultry under stressful conditions (Brien et al., 2013). In their second experiment, Briens et al. (2013) found that the apparent digestibility of total Se (ADSe) significantly increased when organic Se sources were utilized at inclusion rates of 0.3 mg Se/kg complete feed, particularly when HMSeBA was used – 46% vs. 49% for SeYeast and HMSeBA, respectively. The ADSe for sodium selenite was 24%; however, this great deviation from the apparent digestibilities of the organic Se sources can be partly explained by their differences in absorption (Briens et al., 2013). While selenite undergo passive intestinal absorption, SeMet is absorbed in the intestine via active transport (Wolffram et al., 1985).

Deviations in the Absorption of Se Sources

While both inorganic as well as organic forms of Se are readily absorbed with an average efficiency around 80% (ranging between 70-90%), both the level of absorption as well as the utilization of Se have been shown to differ between the various chemical forms of Se (Dalgaard et al., 2018; Sunde, 1997). Some studies, on the other hand, have shown that the absorption efficiency of selenite does not surpass 60% (Stewart et al., 1987). Nonetheless, studies since the late 1970s have provided evidence that, despite differences in absorption, both selenite and SeMet are readily available for the production of selenoenzymes – as was observed by Pierce and Tappel (1997) when they noted that both forms of Se were promptly available for the synthesis of glutathione peroxidase in rat tissues.

As described above, inorganic and organic sources of Se vary in their manner of absorption. Although some investigations have attributed this discrepancy to observed differences in bioavailability or Se deposition into tissues, previous studies have provided evidence that sodium selenite and selenate are absorbed almost as efficiently as SeMet (Combs and Combs, 1984). However, Slupczynska et al. (2018) reviewed a multitude of publications that attributed differences in Se bioavailability to variations in absorption pathways. According to Schrauzer and Surai (2009), this argument is likely plausible since SeMet is able to directly reach the amino acid pool, whereas both selenite and selenate must undergo chemical transformations during absorption that is dependent upon either cysteine or glutathione availability. While selenite is able to pass through the intestinal wall once it is either converted to selenodicysteine or selenodiglutathione, depending on which transporter molecule it interacted with, selenate must be actively transported through the small intestine's brush-border membranes (Würmli et al., 1989; Schrauzer and Surai, 2009). While the selenite-exchangeable pool consists of a variety of Se species, SeMet is not one of them, and thereby provides an explanation for why the selenite-derived Se species are excreted through the urine, feces, or exhaled following methylation rather than retained by the body (Schrauzer and Surai, 2009). Indeed, several studies have shown that SeMet is retained far more efficiently than either selenite or selenate as it is non-specifically incorporated into proteins (Fairweather-Tait, 1997; Habibian et al., 2015). Thus, the form of Se in the diet is a significant determinant of its retention levels.

Metabolism of Selenocompounds: Selenoproteins, Reactive Oxygen Species, and Toxicity

It is important to note that SeMet is the natural dietary form of Se in animal diets. Upon its ingestion and subsequent absorption, SeMet enters the methionine pool of the body by way of intestinal methionine transporters; however, while it can be non-specifically incorporated into proteins such as albumin via this route, the other metabolic fate of SeMet is its breakdown via the methionine cycle and the transsulfuration pathway (McConnell and Cho, 1967; Burk and Hill, 2015; Dalgaard et al., 2018). It is this evidence that SeMet serves as a non-specific form of Se, along with observations that it is not impacted by specific Se metabolic processes, which corroborates its function as the storage form of Se (Surai and Fisinin, 2014). Nonetheless, all dietary forms of Se are required to undergo conversion into an available form of Se prior to the de novo synthesis of SeCys that gives rise to selenoproteins (Rayman, 2008; Dalgaard et al., 2018). As described by Rayman (2004), SeMet is trans-selenated to a transient form of selenocysteine that then undergoes conversion by a β-lyase to inevitably form the intermediate hydrogen selenide (H_2Se) – a form of Se that is available for selenoprotein synthesis (Burk and Hill, 2015). Hydrogen selenide can then be converted to selenophosphate ($HSePO_3^{2-}$) by the enzyme selenophosphate synthetase (Rayman, 2004). At this point, tRNA-bound serinyl residues react with selenophosphate to give rise to SeCys-bound tRNA. This step allows for the co-translational insertion of SeCys at loci encoded by UGA codons; indeed, as is noted in Figure 1.1., this is this last step that ultimately leads to the production of selenoproteins (Berry et al., 1991; Combs, 2001; Rayman, 2004). It is significant to acknowledge that no free pool of SeCys exists in the cell – SeCys incorporation into selenoproteins can only be accomplished via newly synthesized SeCys (Surai, 2006). SeCys is thus recognized as the 21st amino acid because of this highly specific selenoprotein translation pathway that is designed to withstand the reactivity of Se in order to fortify proper SeCys synthesis (Allmang et al., 2009; Arner, 2010; Dalgaard et al., 2018).

While high intake levels of SeMet drive the accumulation of Se in tissues and thus the synthesis of selenoproteins, the breakdown of these SeMet-containing proteins additionally feeds the free methionine pool by releasing SeMet upon degradation (Burk and Hill, 2015). Indeed, the availability of Se thereby regulates the synthesis of selenoproteins, which is particularly important when Se availability is limited as the synthesis of some selenoproteins will be downregulated at the cost of sufficient synthesis of others (Seyadali and Berry, 2004). On the opposite end of the spectrum, excess Se intake, particularly in the form of either sodium selenate or selenite, can also yield undesirable results. As Figure 2.1. depicts, oxidized forms of selenate and selenite are reduced to hydrogen selenide, and when this occurs in excess, the subsequent oxidation of hydrogen selenide results in enhanced generation of reactive oxygen species such as superoxide (Combs, 2001; Rayman, 2004).

Hydrogen selenite is considered to play a central role in the materialization of toxicity. Indeed, as discussed above, hydrogen selenite can produce reactive oxygen species through its reaction with dioxygen and thiols and thereby give rise to oxidative stress, and subsequently, DNA damage (Letavayova et al., 2006; Plateau et al., 2017). A variety of studies in the last decade have used Saccharomyces cerevisiae to show how DNA repair pathways protect yeast cells against selenide and selenite-induced toxicity (Peyroche et al., 2012; Seitomer et al., 2008). In fact, both of these studies by Peyroche et al. (2012) and Seitomer et al. (2008) showed that mutations to genes involved in the homologous recombination and DNA damage checkpoints were hypersensitive to selenide and selenite, respectively (Plateau et al., 2017). These findings gave insight to the means by which toxicity can occur, primarily suggesting that DNA double-stranded breaks may serve as potential cause. On the other hand, Seitomer et al. (2008) observed that with the loss of many of the genes involved in DNA damage and oxidative stress pathways, there was little change in tolerance to SeMet treatment. Lazard et al. (2015) further explained that the basis of SeMet toxicity is less defined and likely deviates from the mechanisms involved in the toxicity of inorganic selenium; in fact, Malkowski et al. (2007) found that cytotoxic compounds that derive from SeMet likely serve as the driving factor of toxicity as opposed to SeMet itself.

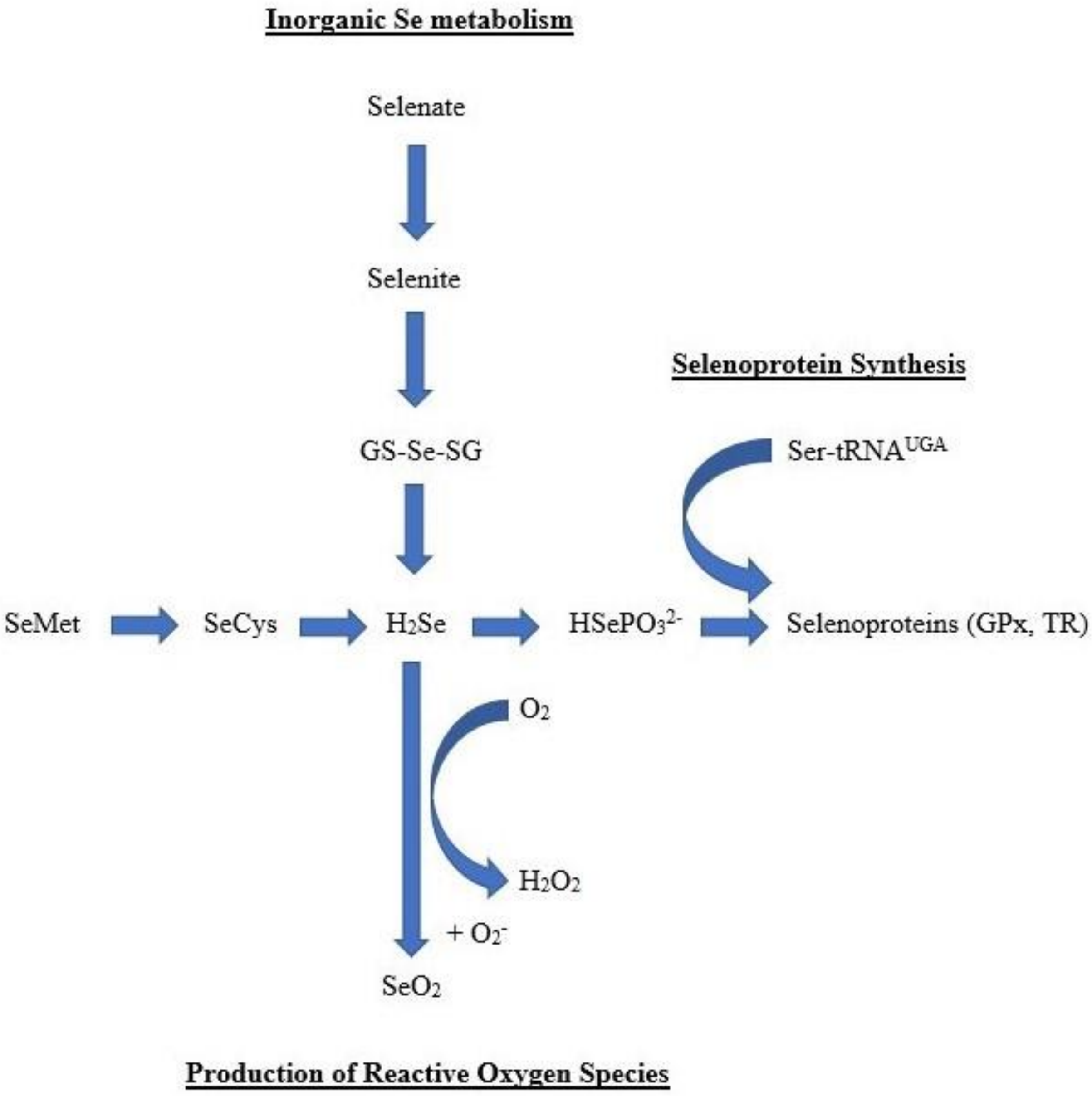

Figure 2.1. Metabolic fates of L-selenomethionine (SeMet) as described and adapted by Combs (2001) and Rayman et al. (2004). SeMet, selenomethionine; SeCys, selenocysteine; Gs-Se-SG, selenodiglutathione; GPx, glutathione peroxidase; TR, thioredoxin reductase.

Nanoparticles: Inorganic and Organic Forms of Trace Minerals

While trace mineral supplementation has been achieved using inorganic sources of trace minerals in the past, the extent of partial or total replacement of these inorganic sources with organic sources have recently been under thorough investigation (Manangi et al., 2015). One of the arguments for the use of organic sources of trace minerals is that they are presumably better-absorbed and thereby increase the mineral content available to the animal (Zamani et al., 2005). Under such conditions, organic trace mineral supplements would allow for the physiological requirements of the bird to be met at lower dietary intake levels than those that are necessary when inorganic sources are utilized; however, the lack of cost-effectiveness of this approach makes the switch from inorganic to organic sources of trace minerals less feasible (Navidshad et al., 2016). The focus upon inorganic nanoparticle supplementation within the last decade has provided some promise. While this form has been shown to be more bioavailable due to the ease with which it is absorbed, there are a variety of production traits that nanoparticles have been described as improving. As stated by Patra and Lalhriatpuii (2019), the ability of trace mineral nanoparticle supplementation to alter various egg and bone quality traits, antioxidant enzyme activities, lipid peroxidation metabolites, and tibia bone strength to a greater degree than that which is observed with large particles of inorganic sources indicates that these nanoparticles may play a role in reinventing how we approach mineral requirements. In other words, the different sources of trace minerals that have been explored in the last couple of decades have each had individual impacts on the dietary requirements of the bird due to their relative bioavailabilites; therefore, the next few decades of research are sure to continue focusing upon how each of these forms impacts bird performance and how the most cost-effective approach can be reached by either fully replacing one form in the diet with another – or establishing a balancing act of one form with another that may lessen the burden of cost (Navidshad et al., 2016).

Recent studies have discovered that supplementation with Se nanoparticles (sodium selenite NPs) at inclusion levels of 0.3 mg/kg of the diet in broiler chickens led to increased serum Se content, glutathione peroxidase activity, and glutathione peroxidase gene expression in the liver compared to both inorganic (sodium selenite) and organic selenium supplementation (Boostani et al., 2015; Patra and Lalhriatpuii, 2019). While Se nanoparticles have been shown to increase selenoenzyme activity similar to SeMet, there are a number of other advantages including: low toxicity, substantial specific surface area, and high catalytic efficiency (Pelyhe and Mezes, 2013; Skalickova et al., 2017; Surai et al., 2017). Again, however, the main drawback is how the use of elemental Se (inorganic forms of Se) can participate in SeCys synthesis and thus sufficient selenoprotein production (Surai et al., 2018). In correlation with this, the argument of low toxicity is strong, but Se toxicity is no longer a prominent issue in poultry nutrition (Surai et al., 2018). A study by Cai et al. (2012) provided promising results when the authors observed that the use of Se nanoparticles at 0.30 mg/kg of feed significantly boosted glutathione peroxidase activity, decreased serum malondialdehyde content, and increased the inhibition of free radicals in broilers. Although there exists some evidence that Se nanoparticles can positively influence poultry performance, its usefulness as a supplement, particularly in comparison to organic sources of Se, requires further investigation.

Selenium in Antioxidant Defense and Immune Response

A principal area of interest with respect to trace mineral supplementation is the manipulation of the bird’s antioxidant system in order to ensure that performance is optimized under physiological or environmental stress conditions. Perhaps one of the most prominent environmental stressors currently facing poultry husbandry is heat stress (Habibian et al., 2015). As described by Mujahid et al. (2005), imperative traits that govern the economic prosperity of the poultry industry, such as growth rate, egg production, and hatchability are highly susceptible to the negative consequences of heat stress. Several studies have shown that poultry exposed to high environmental temperatures that exceed 30 degrees Celsius often demonstrate elevated levels of free radical production as well as concomitant reductions in vitamins and minerals, such as vitamin E, A, and C, that otherwise play significant roles in the antioxidant system (Mujahid et al., 2006; Ghazi et al., 2012). Indeed, under such environmental conditions, it is evident that birds are subjected to both heat stress as well as oxidative stress. While there are a multitude of means by which the antioxidant systems of poultry can be regulated, mitigating the consequences of a stress response through the synthesis of antioxidant enzymes is one of the most promising avenues. Throughout literature, it has been established that the enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase are the cell's first line of antioxidant defense (Surai, 2002). While vitamin E has often been the central focus of the antioxidant system, it does not work alone in its removal of free radicals and detoxification of hydroperoxides; in fact, Se-dependent GSH-Px is also pivotal to these processes (Surai, 2002). An early study by Cheng et al. (1999) showed that despite high dietary levels of vitamin E, the role of cellular GSH-Px in the protection of mice from acute oxidative stress could not be replaced by other vitamins or minerals, as this enzyme depends on sufficient Se status (Surai, 2002). Since dietary Se is critical to the regulation of GSH-Px activity, it is therefore crucial to bird's ability to alleviate oxidative stress. Thus, many investigations into potential mediators of heat stress in poultry involve the supplementation of Se in various forms and inclusion levels.

One of the primary means by which oxidative stress is currently measured in poultry industry is through the evaluation of malondialdehyde (MDA) content in specific tissues since it is the principal product of polyunsaturated fatty acid (PUFA) peroxidation (Habibian et al., 2015). Indeed, Wang et al. (2009) described more than a two-fold increase in MDA content in the skeletal muscle of broiler chicks that were exposed to heat stress compared to those raised under thermoneutral conditions; however, additional studies have shown that MDA concentrations are reduced in heat-stressed broiler chicks upon supplementation with Se at 0.3 mg/kg of complete feed (Xu et al., 2014). While Ghazi et al. (2012) also evaluated MDA concentrations in the breast muscles of heat-stressed birds, the authors additionally assessed superoxide dismutase and glutathione peroxidase activities, whose significance to the antioxidant system was previously mentioned. However, although Ghazi et al. (2012) found that MDA concentrations increased by 2.7-fold in heat-stressed birds relative to control birds, SOD activity was enhanced while glutathione peroxidase activity was not significantly impacted. These results imply that the heat-stressed birds experienced the first state of antioxidant enzyme changes, evidenced by the manipulation of superoxide dismutase activity; however, this line of defense against free radical production did not employ the activation of glutathione peroxidase (Ghazi et al., 2012; Habibian et al., 2015). SOD activity is particularly crucial to the antioxidant system because it catalyzes the dismutation of the superoxide radical; thus, SOD serves as an additional biological marker of oxidative stress. On the other hand, Ghazi et al. (2012) found that when heat-stressed birds were either supplemented with 0.5 or 1.0 mg/kg of Se, the activity of glutathione peroxidase was upregulated and the concentration of MDA in the breast muscles of those birds was significantly less than heat-stressed birds that were not supplemented with these levels of Se. Sahin et al. (2002) reported similar results of reduced MDA content in the serum and liver of heat-stressed quail that were supplemented with Se. It is important to note, however, that the results of similar investigations do not agree with the beneficial effects of Se observed by Ghazi et al. (2012). Indeed, two notable studies in broilers showed that dietary Se levels did not have a significant impact on feed efficiency or body weight gain in heat-stressed birds (Niu et al., 2009; Habibian et al., 2014). Therefore, further investigations are necessary in order to elucidate the effects of dietary Se supplementation, and its respective form, on the performance of heat-stressed laying hens.

Summary

The current aim of trace mineral investigations with respect to Se is to identify the most cost-effective and physiological advantageous source of Se feasible for commercial utilization. While an array of different inorganic and organic Se products are presently employed in poultry nutrition, there is growing evidence that the storage form of Se, SeMet, provides the greatest benefit to the bird relative to sodium selenite and SeYeasts; however, the use of pure SeMet presents its own disadvantages, despite its superior Se retention sufficiency – a quality that is crucial antioxidant status. Recently, the development of a selenomethionine hydroxy analogue (HMSeBA), called Selisseo®, has provided early promise as a potential Se source for use on a global production scale. In light of the matter that this HMSeBA product contains nearly 100% SeMet, and has been evidenced as possessing greater bioavailability relative to other dietary forms of Se, it serves as a front-runner for poultry nutritionists. However, due to the multitude of both inorganic and organic Se products, including the recent developments regarding Se nanoparticle supplementation, further research analyzing these different dietary forms of Se is necessary to provide a better picture of each products' fitness with respect to one another.

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CHAPTER 3

DIETARY INCLUSION OF AZOMITE IMPROVES FEED EFFICIENCY IN BROILERS AND EGG PRODUCTION IN LAYING AND BROILER BREEDER HENS [1]

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[1] Juzaitis-Boelter, C. P., A. P. Benson, M. K. Jones, A. J. Davis, and J. Ferrel. To be submitted to *Poultry Science*.

ABSTRACT

The dietary inclusion of aluminosilicates has been reported to enhance pellet quality, improve feed mill throughput, bind toxins, improve feed efficiency, and promote immunological function across a variety of production systems. AZOMITE® is a product marketed as a hydrated sodium calcium aluminosilicate containing macro and trace minerals, and rare earth elements, and the potential benefits of its dietary inclusion in broiler, layer, and broiler breeder diets was investigated. In a battery study, broilers were fed diets containing 0, 0.125, 0.250 or 0.500% Azomite from 0 to 21 days of age. Laying hens were fed a control diet or this diet supplemented with 0.25% Azomite from 54 through 98 weeks of age, with the hens fed a standard molting diet or this diet supplemented with 0.25% Azomite from 71-72 weeks of age. Broiler breeder hens were fed a control diet or this diet supplemented with 0.25% Azomite from the onset of photostimulation at 21 weeks of age through 65 weeks of age. All 3 dietary inclusion rates of Azomite improved ($P < 0.05$) the feed to body weight gain ratio in broilers fed these diets relative to broilers fed the control diet. In laying hens total marketable eggs, and in broiler breeder hens total settable eggs were increased ($P < 0.05$) with the dietary inclusion of Azomite by 8 and 9 eggs per hen, respectively. The inclusion of dietary Azomite also improved apparent Ca and P digestibility in broilers and tibia ash content in laying hens. The results indicate the dietary inclusion of Azomite in poultry diets improves bird performance.

Key words: tibia ash, molt, Ca and P apparent digestibility

INTRODUCTION

AZOMITE® (AZ; Azomite Mineral Products, Nephi, UT), can be described as a lightly altered vitric, poorly welded dacitic tuff of solidified volcanic igneous rock origin. Volcanic tuff is a rock made from consolidated volcanic ash of small particles, mainly volcanic glass, and other eruptive debris, such as large mineral fragments. Alteration by exposure to water near the surface of the Earth can also produce minerals such as aluminosilicate clays and calcite (calcium carbonate), in place of some of the original material. Thus, AZ is a complex mixture of different kinds of mineral matter, and has been shown to contain measurable amounts of some 74 different elements. Therefore, AZ is a hydrated sodium calcium aluminosilicate that differs from other absorbent aluminum phyllosilicate clays such as calcium bentonite which only consists of 15 additional elements. All of the rare earth elements (REEs), but promethium which is not typically found in the Earth's crust (Hu et al., 2006), are found in AZ. The concentration of REEs in AZ is 530 mg/kg.

Aluminosilicate-based clays have been added to animal diets to improve animal performance and previous research indicates that the clays can bind toxins (Kubena et al., 1998; Chen et al., 2014), slow gastrointestinal transit time for better digestion (Quisenberry, 1968), increase intestinal villi surface area (Wawrzyniak et al., 2017), enhance immunity (Jarosz et al., 2017), and decrease ammonia emissions in poultry litter (Prasai et al., 2017). Luna, et al., (2016) and Wlazlo et al., (2016) reported that the addition of aluminosilicate clays as a litter amendment also reduced ammonia production. Previous research in poultry with rare earth elements indicates that their dietary addition will improve egg production, hatchability of eggs, and feed efficiency in laying hens as reviewed by Lei and Xueying (1997). The addition of REEs to broiler diets has been reported to improve body weight gain (BWG; He et al., 2010).

Although the dietary inclusion of AZ might provide the benefits seen previously with the individual addition of aluminosilicate clays or REE's in poultry production, research involving AZ in poultry is limited. Within feed mills, AZ is used for its anticaking properties and recently Tillman at al., (2020) reported that the inclusion of AZ at 0.25% and 0.50% improved pellet production rates when meat and bone meal (4%) and/or dried distillers grains with solubles (8%) were used in the diet. Therefore, the goal of the current research was to evaluate the potential production benefits of including dietary AZ when feeding broilers from d of hatch until 21 d of age, laying hens from 54 to 98 wk of age with a molting diet fed from 71 through 72 wk of age, and broiler breeder hens from 21 to 65 wk of age.

MATERIALS AND METHODS

All animal procedures were approved by the University of Georgia Animal Care and Use Committee.

Experiment 1

The aim of the first experiment was to evaluate the performance of broilers from 0 to 21 d of age fed a starter diet supplemented with AZ at 0, 0.125, 0.250, and 0.500% of the diet (Table 3.1). Five hundred, male by-product broiler chicks from a female parent stock (Cobb 500 fast feathering) obtained from the Cobb hatchery in Cleveland, GA, were raised in thermostatically controlled, electrically heated battery brooder cages with wire floors. Chicks had free access to water and the starter diet. Prior to placement, the chicks were sorted according to weight profiles and those with extreme weights or physical abnormalities such as open navels were discarded. The remaining chicks were assigned to experimental groups in order to achieve similar weight distributions among all pens and minimize variation.

The dietary treatments were equally distributed and randomized across three battery brooders each equipped with 24 pens to create 18 replicate pens for each dietary treatment. Each replicate pen consisted of 5 chicks. Individual pens measured 98 cm long by 35 cm wide by 23 cm high. A computerized controller for the room housing the batteries regulated a gas-fired furnace, exterior evaporative cooling system for intake air, 46-cm ceiling circulation fan, and 2 exhaust fans, one measuring 53-cm, and the other one measuring 26-cm, at the end of the room for heating, cooling, and ventilation. Ambient temperature was set to 34 °C on d 0 and decreased by 0.28 °C each d. For the duration of the study, light intensity was 20 lux for 24 h a d. Body weight and total feed consumption on a pen basis were determined every 7 d.

In order to determine apparent calcium and phosphorus digestibility, the exact amount of feed consumed during the last 48 h of the experiment was determined and total feces were also collected in clean stainless steel pans for each pen during this time. To ensure clearance of the digestive tract of food, the feeding troughs were removed 12 h prior to the start of this feeding period and 12 h before the end of the study when the feces were collected. On the final day of the experiment, after weighing the birds and feed, blood samples were collected from the brachial vein of the control and the 0.500% AZ-supplemented birds to obtain serum samples. Serum was frozen at -80°C.

Experiment 2

This experiment aimed to evaluate the potential production benefits of the dietary inclusion of AZ at 0.25% of the diet in laying hens from 54 through 98 wk of age, with the additional stress of a molting period from 71 through 72 wk of age. Egg production and hen BW were monitored in 120 individually caged, 50-wk-old, Hy Line W-36 White Leghorn laying hens for a pre-experimental period of 4 wk. Cages were in 3 tiered batteries with each battery containing 48 cages. Each cage had a sloped floor for egg displacement from the interior of the cage; thus, the height of the cage was 41 cm in the back and 46 cm in the front, while cage width and depth were 33 cm and 46 cm, respectively. Each cage was equipped with a nipple drinker and individual trough feeder. The hens were housed in an environmentally controlled room equivalent to the one described in Experiment 1 with a set temperature of 22.5°C. The daily lighting schedule provided 16 h of light and 8 h of dark.

At the end of the third wk, the 96 birds with the greatest egg production and egg specific gravity values were selected and divided into 2 treatment groups and 2 batteries. Each treatment group had 3 replicate rows of 8 individually caged hens per battery (n = 6 replicate rows per treatment). The hens had a 1-wk period to re-assimilate to this distribution prior to the commencement of the experiment at 54 wk of age. The hens were distributed such that the 12 rows of hens did not differ in body weight profile, egg production or egg specific gravity at the start of the experiment.

The laying hens were fed a standard corn-soy diet supplemented with 0.25% Solka-Floc (control diet; International Fiber Corporation, North Tonawanda, NY) or 0.25% AZ (Table 3.2) from 54 to 71 and 73 through 98 weeks of age. From 71 through 72 weeks of age the hens were fed a molting diet (Table 3.2) containing 0.25% Solka-Floc or 0.25% AZ.

Every 4 wk during the 44 wk experimental period, all hens were individually weighed and total feed consumption per replicate row of hens was determined. In addition, blood was collected from the brachial vein of half of the laying hens (24 per treatment) on a rotating basis every 4 weeks. Serum from these blood samples was stored at -80° C. Daily egg production and weekly egg weights were recorded for each bird and each replicate row of hens, respectively. Hen-housed and hen-day egg production were calculated weekly from daily egg counts. Eggs were manually collected once per day, and at the time of egg collection, all eggs were classified as normal (marketable eggs), cracked, double-yolked, misshapen, membrane, or dirty. Every 4 wk, 2 d-worth of egg production was saved for specific gravity measurement following procedures similar to Phillips and Williams (1944)**.**

At the conclusion of the experiment, a 3-cm segment from the top of the duodenal loop and the right tibia were collected from 24 hens from each treatment with the greatest egg production for histology and bone ash determination, respectively. Each collected segment of the duodenum was flushed with 10% formalin (3.7% formaldehyde) prior to being submerged in 10% formalin. After formalin fixation, the samples were embedded in paraffin wax, sectioned at 4 microns, routinely stained with hematoxylin and eosin and cover slipped. Slides were examined by light microscopy and photomicrographs were taken using a Leica (Wetzlar, Germany) DF550 model camera with LAS V4.8 imaging software. For each sample, 3 villi and crypts were measured with the values obtained averaged. Tibias were cleaned of adhering tissue, defatted, and dried before ash determination following the AOAC (2011) method.

Experiment 3

The aim of this experiment was to evaluate the influence of the dietary addition of AZ on the performance of broiler breeder hens from the time of photostimulation at 21 wk of age through 65 wk of age. From 1 d of age, Cobb 500 fast feathering pullets and cockerel were reared as previously described (Gibson et. al., 2008).

At 20 wk of age, all pullets were individually weighed. The pullets were matched by weight into 38 categories for placement into the laying pens. To ensure that the weight distribution in each pen was similar, a pullet from each of the 38 weight categories was then randomly selected and distributed to a laying pen. There were 18 laying pens, and each contained 38 pullets and 4 roosters. Each pen measured 3.65 × 2.75 m, and the floor space of each pen consisted of two-thirds pine shavings litter and one-third elevated slats. Each pen had one 6-hole nest box located on the slatted area and was equipped with 10 nipple drinkers. Hens and roosters were hand-fed with plastic feeder pans in the laying pens. Each pen contained 3 hen feeder pans that were fitted with rooster exclusion grills. The feeding system provided 8.4 cm of feeder space per hen. Males were provided their own feeder pan, which was elevated in height in order to prevent females from consuming their feed. Each rooster had 25.9 cm of feeder space. The male-to-female ratio was maintained throughout the experiment by replacing dead males from a pool of extra males. Photostimulation occurred at 21 wk of age by providing 14 h of light (lights on at 0630 h), and this photoperiod was maintained until the end of the experiment when the birds completed their 65 wk of age.

Prior to the start of the experiment at 21 wk of age, the broiler breeders were being fed a developer diet. At the initiation of the experiment, 9 of the replicate breeder pens continued on this developer diet supplemented with 0.25% Solka-Floc while the birds in the remaining 9 pens were fed this diet supplemented with 0.25% AZ (Table 3.3). At 24 wk of age, the broiler breeders were switched to laying diets for the rest of the experiment that contained 0 or 0.25% AZ (Table 3.3). During the entire experimental period the birds were fed on daily basis at 06:30 h. The amount of feed provided to the birds during the breeding period was based on BW and egg production, as suggested by the guidelines of the primary breeder. All of the hens were individually weighed at the start of the experiment and at wk 26, 30, 35, 39, 43, 51, and 61 wk of age. For the other weeks of the experimental period, the hens from 3 of the 9 pens for each treatment were weighed on a rotating basis every wk.

Eggs were manually collected 2 times per d, and egg production was calculated weekly from daily egg counts. At the time of egg collection, all eggs were classified as normal (settable eggs), cracked, double-yolked, misshapen, membrane, or dirty. Every 4 wk, collected eggs were saved for specific gravity analysis. Settable eggs produced in each room were weighed every other week. The specific gravity of settable eggs was measured following procedures similar to Phillips and Williams (1944), when the hens were 30, 35, 39, 44, 48, 53, 58, and 65 wk of age.

Ninety settable eggs from each pen were incubated (Natureform Hatchery Systems, Jacksonville, FL) every 3 wk when the hens were 26 to 59 wk of age. Eggs were collected and stored between 18.3 and 19.9°C for up to 7 d before each incubation period. Eggs were candled on d 14 of incubation, transferred for hatching on d 19 of incubation, and hatched on d 21 of incubation. Eggs were incubated at 37.8°C with 53% relative humidity from d 0 through 18, and then at 37.2°C with 70% relative humidity from d 19 to 21. During candling and transfer and after hatching, eggs were characterized as being infertile, cracked, contaminated, or containing early dead embryos (less than 14 d), or late-dead embryos (15 to 21 d). Eggs that were cracked during transfer were removed from the data set.

Alpha-1-acid glycoprotein and IgY ELISA

The alpha-1-acid glycoprotein (AGP) content of serum samples collected at the end of broiler experiment (Experiment 1) and when the laying hens (Experiment 2) were 54, 70 and 73 wk of age, was determined using the ABCAM Chicken Alpha-1-acid Glycoprotein Sandwich ELISA (Cambridge, MA) following the manufacturer’s protocol. The IgY content of serum samples collected from the laying hens at 54 and 70 wk of age was determine utilizing an ABCAM Chicken IgY Sandwich ELISA Kit (Cambridge, MA). A SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA) was used for the colorometric analysis of the samples for each ELISA.

Statistical analysis

Data from each experiment were subjected to ANOVA according to the General Linear Model (GLM) with dietary treatment as the factor in the statistical model in experiments 1, 2, and 3 and used to detect significant weekly or overall experimental period differences between the control and AZ treatments in Experiments 2 and 3. In Experiment 1, Tukey's multiple-comparison procedure was used to detect significant differences among individual dietary treatments (Neter et al., 1990). Differences were considered significant when $P < 0.05$. All statistical procedures were completed with the Minitab statistical software package (Release 16, State College, PA).

RESULTS

Experiment 1

The addition of 0.125, 0.250, and 0.500% AZ to a control broiler starter diet improved the feed to gain ratio at 21 d of age (Table 3.4). Apparent calcium and phosphorus digestibility were improved and the blood AGP concentrations were reduced in broilers fed a diet containing 0.50% AZ relative to broilers fed a control diet with no AZ (Table 3.5).

Experiment 2

Feed intake, BWG, and BW loss during the molting period did not differ between the hens fed the control or molting diets, and the hens fed these diets supplemented with AZ (Table 3.6 and Table 3.7). Over the entire experimental period, the addition of AZ improved total egg and marketable egg production (Table 3.8). In particular, the hens fed the AZ-supplemented diet returned to lay at a quicker rate after molt than the control hens (Figure 3.1). Although not significant, cracked egg production and total unmarketable egg production were numerically decreased by about half for the entire experimental period for the hens fed the diet supplemented with AZ (Tables 3.9). Egg weight was unaffected by the addition of AZ to the laying hen diets (Figure 3.2). The specific gravity of eggs produced by the hens fed a diet containing AZ was sometimes less than the eggs produced by the control hens (Table 3.10). However, tibia ash percent was greater at the conclusion of the experiment in the hens that had been fed diets supplemented with AZ (Table 3.11). Blood AGP levels were not affected by dietary AZ supplementation, but AGP levels were greater at the end of the molting period than prior to molt in both the control and AZ hens (Table 3.12). The only mortality during the experiment was one control hen at 94 wk of age.

Experiment 3

Throughout the experiment, there was no difference in BW between the broiler breeder hens fed the control diet or this diet supplemented with AZ (Table 3.13). Weekly hen housed egg production did not differ (Figure 3.3) between the 2 dietary treatments. Overall, settable egg production was significantly greater for the broiler breeder hens fed the AZ- supplemented diet (Table 3.14). Egg weight did not differ throughout the experiment between the two dietary treatments (Table 3.15). Specific gravity values of the eggs only differed at 53 wk of age when the eggs from the hens fed the diet supplemented with AZ had a lower value than the eggs produced by the control hens (Table 3.16). Overall, hatchability and fertility did not differ between the eggs produced from the hens fed the control diet or this diet supplemented with AZ (Table 3.17). However, the overall number of chicks that pipped, but did not hatch (in shell mortality) was greater for the eggs from the hens fed the diet supplemented with AZ (Table 3.17). At the start of the experiment, the average number of hens per pen was 38 for each treatment. With subsequent mortality, the average number of hens was 34 and 32 hens per pen for both the control and AZ treatment at 49 and 65 wk of age, respectively.

DISCUSSION

The current research indicates that adding AZ to broiler, laying hen and broiler breeder diets has positive effects on bird performance, but the specific mechanisms by which AZ improves performance was not determined. In experiment 1, the apparent digestibility of Ca and P was improved in broilers fed a diet supplemented with 0.5% AZ. In a subsequent experiment (not presented), the true digestibility coefficients of Ca and P were determined at 42 days of age in broilers that had been fed from d of age diets containing either 0 or 0.125% AZ (20 broilers per treatment). The digestibility coefficient of Ca was 49 and 68 percent ($P < 0.05$) in the control and AZ broilers, respectively while the digestibility coefficient of P was 57 and 68 percent ($P = 0.07$), respectively. If the increased Ca and P digestibility seen with AZ in broilers also occurs in laying hens it may have contributed to the laying hens fed diets supplemented with AZ having increased tibia ash values at the end of the experiment relative to control-fed hens.

Although the specific gravity of eggs produced by the laying hens fed a diet containing AZ was at times significantly less than the eggs produced by the control hens (Table 3.10), it is important to keep in mind these hens produced over 8 more marketable eggs per hen than the control hens without a reduction in egg size (Figure 3.2). Thus, the AZ-fed hens had a greater calcium demand for egg shell formation, and while not significant, numerically produced half as many cracked eggs relative to the hens fed the control diet. Similarly, the broiler breeder hens fed the diet supplemented with AZ produced about 9 more settable eggs than the control fed hens, with no decrease in egg size and only a decrease in egg specific gravity detected at one-time point in the experiment. Reka et al., (2018) fed laying hens a control diet or this diet supplemented for 2 months with either a combination of 100 mg/kg lanthanum and 150 mg/kg cerium or 200 mg/kg lanthanum and 200 mg/kg cerium. The combination of these 2 specific REE at the low dose increased blood Ca and P levels in the laying hens when measured at 4 and 8 wk after the start of the experiment. Laying hens fed the high dose of these 2 specific REE had increased blood Ca and P levels at 4 wk, but not at 8 wk. Bölükbaşı,et al. (2016) reported that the addition of cerium oxide to laying hen diets at 100 mg/kg, but not at 200, 300, or 400 mg/kg, increased blood Ca and P levels. However, laying hens fed diets containing 0, 100, 200, 300 or 400 mg/kg lanthanum oxide did not differ in serum Ca and P levels (Durmuş and Bölükbaşı, 2015). This previous research suggests that the REE content of AZ may contribute to improving Ca absorption and warrants subsequent research in laying hens where improved Ca absorption can impact egg shell quality and bone health.

Based on previous research, the REE contained in AZ might also be playing a role in the increased egg production seen in the current experiment with laying hens and broiler breeder hens. As reviewed by Lei and Xueying (1997), the addition of REE to laying hen diets has improved egg production. Subsequently, specific REE have been investigated for their potential role in improving egg production in laying hens. Egg production in laying hens was increased relative to control hens when cerium oxide was added to the diet at 100, 200, 300, or 400 mg/kg (Bölükbaşı et al., 2016). For laying hens fed diets containing 0, 100, 200, 300, or 400 mg/kg lanthanum oxide egg production relative to the control hens was only increased in the hens fed the 400 mg/kg dose (Durmuş and Bölükbaşı, 2015). The combination of 100 mg/kg lanthanum and 150 mg/kg cerium and 200 mg/kg lanthanum and 200 mg/kg cerium each improved egg production in laying hens (Reka et al., 2019).

Acute-phase proteins are primarily produced and secreted by the liver. As reviewed by O'Reilly and Eckersall (2014) these serum factors mediate the acute phase response to systemic or local perturbations such as inflammation, infection, and stress. One of the major acute-phase proteins is AGP and its serum concentration increases during an acute phase protein response and its production is regulated by a host of mediators such as cytokines and glucocorticoids as reviewed by Fournier et al., (2000). Given the lower serum concentration of AGP in the broilers fed a diet supplemented with AZ in experiment 1, it was hypothesized that AZ supplementation of the laying hen diet might modify the expected rise in serum AGP in laying hens undergoing the stress of a molt. Shakeri et al., (2014) reported that the stress associated with high stocking density was associated with an increase in serum AGP levels, and that food deprivation in broilers (Najafi et al., 2016, 2018) increased the serum AGP concentration and corticosterone levels, but chemically inhibiting the rise in corticosterone during the food deprivation prevented the rise in AGP (Najafi et al., 2018). In the current research, there was an equal rise in the serum AGP concentration in the control and AZ-fed hens from the start to the conclusion of the molting period. However, the hens fed the AZ supplemented diet did return to lay at a quicker initial rate than the control hens (Figure 3.1).

Mounting an acute phase protein response requires energy for maintenance that otherwise could be used for growth in broilers and this may be playing a role in why the control broilers had a greater feed to gain ratio than the broilers fed diets supplemented with AZ in the current research. The improvement in feed efficiency in the AZ birds could be the result of a combination of mechanisms as a result of the complex components inherently within the material. Previous research indicates that various clays or aluminosilicates classified as hydrated sodium calcium aluminosilicate can bind toxins (Kubena et al., 1998; Chen et al., 2014), slow gastrointestinal transit time for better digestion (Quisenberry, 1968) and increase intestinal villi surface area (Wawrzyniak et al., 2017) for potential better absorption of nutrients. Similarly, as reviewed by Panichev (2015) and Wakabayashi et al., (2016), REE has antibacterial and antiviral activities, influences immunity (including anti-inflammatory), and alters hormone production and enzyme activity. As recently reviewed by Tariq et al., (2020) in swine and poultry production and previously by Lei and Xueying (1997) in poultry, the use of individual or combinations of specific REE has been reported to improve feed utilization efficiency. However, the results are not uniform and supplements of individual REE can have negative effects on animal performance which may be related to the over-supplementation and associated toxicity of elevated REE (Pagano et al., 2015; Panichev, 2015).

In summary, the addition of AZ to broiler diets improved feed to gain values through 21 d of age, and the supplementation of laying hen and broiler breeder hen diets improved marketable egg and settable egg production. The results from the current research also suggest that dietary supplementation of poultry diets with AZ may improve Ca and P absorption and utilization. Further research is needed to elucidate the mechanisms by which AZ supplementation of diets improves poultry production efficiency.

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Table 3.1. Composition of the broiler starter diet (Experiment 1)[1].

Ingredient	Diet[1]
	%
Corn	57.625
Soybean meal (48%CP)	32.320
Corn DDGS	3.000
Soybean oil	2.593
Calcium Carbonate	0.646
Defluorinated Phosphate	1.237
Salt	0.353
L-Lysine, HCl 78.8%	0.236
DL- Methionine 99%	0.298
L-Threonine 98%	0.077
Choline Chloride 60%	0.045
Vitamin mix[2]	0.386
Mineral mix[3]	0.075
Solka-Floc[4]	0.500
Quantum blue phytase (5,000 FTU/g)[5]	0.060
Econase XT[6]	0.010
Coban 90[7]	0.050
Calculated analysis	
AME (kcal/kg)	2964
Crude protein (%)	20.090
Calcium (%)	0.950
Available phosphorus (%)	0.480
Digestible total sulfur amino acids (%)	0.857
Digestible lysine (%)	1.127
Digestible threonine (%)	0.733

[1] Starter diet will be fed from d 0 to 21 d of age.

[2] Vitamin premix (DSM custom vitamin premix. DSM Nutritional Products, Inc. Parsippany, NJ) provides the following per kilogram of diet: vitamin A, 5,510 IU; vitamin D_3, 1,100 IU; vitamin E, 11 IU; vitamin B_{12}, 0.001mg; riboflavin, 4.4 mg; niacin, 44 mg; d-panthotenic acid, 12 mg; choline chloride, 220 mg; menadione sodium bisulfate, 3.34 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin mononitrate, 2.2 mg; d-biotin, 0.11 mg; and ethoxyquin, 125 mg.

[3] Trace mineral premix (Southeastern Minerals custom trace mineral mix. Southeastern Minerals Inc. Bainbridge, GA) provides the following in milligrams per kilogram of diet: manganese, 60; zinc, 50; iron, 30; copper, 5; iodine, 1.5; selenium, 0.5. Trace mineral forms were manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, calcium iodate, and sodium selenite.

[4] AZOMITE (Azomite Mineral Products, Nephi, UT) was added at the expense of Solka-Floc (International Fiber Corporation, North Tonawanda, NY).

[5] AB Vista (Plantation, FL)

[6] AB Vista (Plantation, FL)

[7] Elanco Animal Health (Greenfield, IN)

Table 3.2. Composition of the laying hen diets (Experiment 2).

Ingredient	Laying diet[1]	Molting diet[2]
	%	
Corn	56.117	23.000
Soybean meal (48%CP)	27.754	0.000
Soybean oil	3.780	0.000
Limestone	9.785	9.785
Dicalcium Phosphate	1.360	1.360
Salt	0.239	0.235
Sodium carbonate	0.088	0.088
L-Lysine, HCl 78.8%	0.016	0.000
DL- Methionine 99%	0.249	0.000
L-Threonine 98%	0.023	0.000
L-Tryptophan 98%	0.008	0.000
Choline Chloride 60%	0.020	0.020
Quantum blue phytase (5,000 FTU/g)[3]	0.010	0.010
Vitamin mix[4]	0.227	0.227
Mineral mix[5]	0.075	0.075
Solka-Floc[6]	0.25	0.25
Wheat middlings	0	42.950
Soy hulls	0	22.000
<u>Calculated analysis</u>		
AME (kcal/kg)	2867	1875
Crude protein (%)	17.257	10.444
Calcium (%)	4.406	4.436
Available phosphorus (%)	0.505	0.544
Digestible total sulfur amino acids (%)	0.710	0.225
Digestible lysine (%)	0.822	0.378
Digestible threonine (%)	0.589	0.271

[1] Layer diet fed from 54 wk of age to commencement of molting period at 70 wk of age. Hens returned to respective layer diet from 73 wk of age to 98 wk of age.

[2] Layer molting diet fed from 71 through 72 wk of age.

[3] AB Vista (Plantation, FL)

[4] Vitamin premix (DSM custom vitamin premix. DSM Nutritional Products, Inc. Parsippany, NJ) provides the following per kilogram of diet: vitamin A, 5,510 IU; vitamin D_3, 1,100 IU; vitamin E, 11 IU; vitamin B_{12}, 0.001mg; riboflavin, 4.4 mg; niacin, 44 mg; d-panthotenic acid, 12 mg; choline chloride, 220 mg; menadione sodium bisulfate, 3.34 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin mononitrate, 2.2 mg; d-biotin, 0.11 mg; and ethoxyquin, 125 mg.

[5] Trace mineral premix (Southeastern Minerals custom trace mineral mix. Southeastern Minerals Inc. Bainbridge, GA) provides the following in milligrams per kilogram of diet: manganese, 60; zinc, 50; iron, 30; copper, 5; iodine, 1.5; selenium, 0.5. Trace mineral forms were manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, calcium iodate, and sodium selenite.

[64] AZOMITE (Azomite Mineral Products, Nephi, UT) was added at the expense of Solka-Floc (International Fiber Corporation, North Tonawanda, NY).

Table 3.3. Composition of the experimental broiler breeder diets (Experiment 3).

Ingredient	Diet[1]	Diet[2]
		%
Corn	59.750	62.050
Wheat middlings	21.000	7.71
Soybean meal (48%CP)	14.000	16.3
Soybean oil	0.540	2.62
Limestone	1.000	7.37
Defluorinated Phosphate	1.770	0.000
Dicalcium Phosphate	0.000	0.1.73
Salt	0.160	0.190
L-Lysine, HCl 78.8%	0.070	0.260
DL- Methionine 99%	0.500	0.300
L-Threonine 98%	0.080	0.260
Vitamin mix[3]	0.800	0.800
Mineral mix[4]	0.080	0.160
Solka-Floc[5]	0.250	0.250
Calculated analysis		
AME (kcal/kg)	2836	2864
Crude protein (%)	14.483	13.920
Calcium (%)	1.076	3.432
Available phosphorus (%)	0.448	0.420
Digestible total sulfur (%)	0.869	0.669
Digestible lysine (%)	0.642	0.780
Digestible threonine (%)	0.517	0.683

[1] Developer diet fed from wk 21 (onset of photostimulation) until wk 24.

[2] Layer diet fed from wk 24 until wk 65.

[3] Vitamin premix (DSM custom vitamin premix. DSM Nutritional Products, Inc. Parsippany, NJ) provides the following per kilogram of diet: vitamin A, 5,510 IU; vitamin D_3, 1,100 IU; vitamin E, 11 IU; vitamin B_{12}, 0.001mg; riboflavin, 4.4 mg; niacin, 44 mg; d-panthotenic acid, 12 mg; choline chloride, 220 mg; menadione sodium bisulfate, 3.34 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin mononitrate, 2.2 mg; d-biotin, 0.11 mg; and ethoxyquin, 125 mg.

[4] Trace mineral premix (Southeastern Minerals custom trace mineral mix. Southeastern Minerals Inc. Bainbridge, GA) provides the following in milligrams per kilogram of diet: manganese, 60; zinc, 50; iron, 30; copper, 5; iodine, 1.5; selenium, 0.5. Trace mineral forms were manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, calcium iodate, and sodium selenite.

[5] AZOMITE (Azomite Mineral Products, Nephi, UT) was added at the expense of Solka-Floc (International Fiber Corporation, North Tonawanda, NY).

Table 3.4. Body weight, body weight gain (BWG) and feed efficiency of broilers fed diets containing 0, 0.125, 0.250 or 0.500 % AZOMITE (AZ) from 0 to 21 d of age (Experiment 1)^{1}.

Dietary treatments	BW	BWG	Feed to gain	Feed intake	Mortality
			g		%
Control	970 ± 6^{b}	927 ± 6^{b}	1.356 ± 0.008^{a}	1241 ± 11	1.11
0.125% AZ	1004 ± 10^{a}	961 ± 10^{a}	1.316 ± 0.007^{b}	1253 ± 11	4.44
0.250% AZ	991 ± 8^{ab}	948 ± 8^{ab}	1.313 ± 0.007^{b}	1243 ± 10	3.33
0.500% AZ	987 ± 6^{ab}	944 ± 6^{ab}	1.318 ± 0.007^{b}	1254 ± 10	2.22

[1] The values are means ± SEM, n = 18 replicate pens for the dietary treatments. $^{a\text{-}b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 3.5. Apparent Ca and P digestibility and serum alpha 1 acid glycoprotein (AGP) concentration in broilers fed diets containing 0, or 0.500 % AZOMITE (AZ) from 0 to 21 d of age (Experiment 1)[1].

Dietary treatments	Apparent P digestibility	Apparent Ca digestibility	AGP[2]
		%	ug/mL
Control	51.32 ± 1.15^{b}	48.61 ± 1.44^{b}	255 ± 12^{a}
0.500% AZ	54.80 ± 0.83^{a}	56.48 ± 1.53^{a}	224 ± 7^{b}

[1] The values are means ± SEM, n = 18 replicate pens. $^{a\text{-}b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

[2] Alpha 1 acid Glycoprotein.

Table 3.6. Body weight of hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ; Experiment 2)[1].

Hen age	Dietary treatment	
	Control	AZ
wk		g
53 (pre-experiment)	1561 ± 2	1561 ± 2
54	1589 ± 9	1598 ± 10
57	1648 ± 12	1672 ± 18
62	1661 ± 9	1682 ± 19
66	1711 ± 15	1723 ± 15
70	1734 ± 19	1747 ± 17
72	1375 ± 26	1386 ± 9
77	1648 ± 10	1680 ± 17
81	1773 ± 27	1803 ± 19
85	1813 ± 24	1808 ± 30
88	1807 ± 23	1848 ± 20
92	1785 ± 16	1814 ± 10
98	1831 ± 12	1843 ± 18

[1] The values are the means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens.

Table 3.7. Feed consumption of hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ; Experiment 2)[1].

Age	Dietary treatment	
	Control	AZ
wk	g/hen/d	
54-70 to molt	98.51 ± 2.12	100.11 ± 1.25
71-72 (molt)	37.58 ± 3.18	36.55 ± 2.56
73-98 (after molt)	101.07 ± 1.00	102.02 ± 1.12

[1] The values are the means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens.

Table 3.8. Total egg and marketable egg production per hen housed of laying hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ; Experiment 2)[1].

	Total egg production		Total marketable egg production	
	Control	AZ	Control	AZ
		eggs/hen		
Before molt (wk 54-71)	99.50 ± 1.68	100.69 ± 1.04	98.27 ± 1.46	100.07 ± 1.02
After molt (wk 73-98)	135.67 ± 2.67	141.69 ± 1.48	134.65 ± 3.01	141.19 ± 1.58[2]
Total production (wk 54-98)	235.17 ± 2.01	242.39 ± 2.01*	232.92 ± 2.09	241.26 ± 2.02*

[1] The values are means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens. *AZ value for a given parameter differs from control value, ($P < 0.05$).

[2] P-value equals 0.083.

Table 3.9. Unmarketable egg production of laying hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ; Experiment 2)[1].

	Control	AZ
	eggs/hen	
Cracked eggs		
Before molt (weeks 54-71)	1.04 ± 0.34	0.51 ± 0.16
After molt (weeks 73-98)	0.75 ± 0.43	0.34 ± 0.21
Total production (weeks 54-98)	1.79 ± 0.72	0.85 ± 0.36
Soft shell eggs		
Before molt (weeks 54-71)	0.17 ± 0.08	0.11 ± 0.07
After molt (weeks 73-98)	0.27 ± 0.20	0.17 ± 0.05
Total production (weeks 54-98)	0.44 ± 0.23	0.28 ± 0.05
Double yolk eggs		
Before molt (weeks 54-71)	0.02 ± 0.02	0.00 ± 0.00
After molt (weeks 73-98)	0.00 ± 0.00	0.00 ± 0.00
Total production (weeks 54-98)	0.02 ± 0.02	0.00 ± 0.00
Total unmarketable eggs		
Before molt (weeks 54-71)	1.23 ± 0.35	0.63 ± 0.18
After molt (weeks 73-98)	1.02 ± 0.62	0.50 ± 0.20
Total production (weeks 54-98)	2.25 ± 0.90	1.13 ± 0.32

[1] The values are means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens.

Table 3.10. Specific gravity of eggs from laying hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ; Experiment 2)[1].

Hen age (wk)	Control	AZ
53 (pre-experiment)	1.071 ± 0.001	1.070 ± 0.001
54	1.075 ± 0.001	1.074 ± 0.001
57	1.082 ± 0.000	1.079 ± 0.000*
62	1.080 ± 0.001	1.079 ± 0.001
66	1.075 ± 0.001	1.072 ± 0.001*
70	1.076 ± 0.001	1.075 ± 0.000
76	1.079 ± 0.001	1.077 ± 0.001
81	1.081 ± 0.001	1.078 ± 0.001*
85	1.078 ± 0.001	1.077 ± 0.001
88	1.077 ± 0.001	1.074 ± 0.001*
92	1.069 ± 0.001	1.065 ± 0.000*
96	1.074 ± 0.001	1.072 ± 0.000

[1] The values are means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens. *AZ value for a given parameter differs from control value, ($P < 0.05$).

Table 3.11. Tibia ash, duodenum villi height and crypt depth for laying hens at 98 wk of age fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 54 wk of age (Experiemnt 2)[1].

Treatment	Tibia ash	Villi height	Crypt depth	Villi:height : crypt depth
	%	nm		
Control	52.44 ± 0.46	1822 ± 78	167 ± 9	11.19 ± 0.42
AZ	54.48 ± 0.51	1747 ± 62	145 ± 6	12.38 ± 0.51
P-value	0.005	0.458	0.051	0.083

[1] The values are means ± SEM for 24 individual hens for each treatment.

Table 3.12. Alpha 1 acid glycoprotein (AGP) acute phase protein concentration and IgY concentration in serum of laying hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 54 wk of age (Experiment 2)[1].

	AGP		IgY	
Hen age (wk)	Control	AZ	Control	AZ
	mg/mL		mg/ml	
54 (start of experiment)	1.12 ± 0.22^{b}	1.37 ± 0.19^{b}	109 ± 12	131 ± 12
70 (before start of molt)	1.38 ± 0.19^{b}	1.18 ± 0.17^{b}	106 ± 11	114 ± 10
73 (end of molt)	2.75 ± 0.19^{a}	2.54 ± 0.27^{a}	ND	ND

[1] The values are means ± SEM for 24 individual hens for each treatment.

$^{a\text{-}b}$Values within a column with different superscripts for a given parameter differ, ($P < 0.05$).

ND = not determined.

Table 3.13. Body weight of broiler breeder hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 21 (onset of photostimulation) through 65 wk of age (Experiment 3)[1].

	Dietary treatment	
Age	Control	AZ
wk	g	
21 (pre-experiment)	2076 ± 15	2067 ± 15
26	3342 ± 18	3320 ± 18
30	3851 ± 31	3821 ± 39
35	4353 ± 34	4325 ± 37
39	4562 ± 125	4503 ± 42
43	4668 ± 30	4668 ± 30
51	4741 ± 114	4746 ± 75
61	4760 ± 110	4833 ± 64

[1] The values are the means ± SEM of 9 replicate pens of 38 hens per treatment.

Table 3.14. Total egg and settable egg production per hen housed of broiler breeder hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 21 (onset of photostimulation) through 65 wk of age (Experiment 3)[1].

	Control	AZ
		eggs/hen
Total egg production	158.11 ± 5.68	165.57 ± 4.22
Total settable egg production	131.55 ± 4.76	140.48 ± 4.00*
Total cracked egg production	5.52 ± 0.32	5.47 ± 0.36
Total misshaped egg production	1.94 ± 0.13	2.00 ± 0.18
Total double-yolked egg production	0.92 ± 0.11	0.80 ± 0.13
Total dirty egg production	11.76 ± 0.99	11.38 ± 0.37
Total floor egg production	5.87 ± 0.60	5.43 ± 0.60

[1] The values are means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens. *AZ value for a given parameter differs from control value, ($P < 0.05$).

Table 3.15. Egg weight of eggs produced from broiler breeder hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 21 (onset of photostimulation) through 65 wk of age (Experiment 3)[1].

	Dietary treatment	
Age	Control	AZ
wk	g	
27	50.53 ± 0.39	50.51 ± 0.59
29	54.18 ± 0.22	53.84 ± 0.39
31	58.29 ± 0.38	58.15 ± 0.35
33	60.82 ± 0.28	61.18 ± 0.27
35	61.75 ± 0.28	61.93 ± 0.26
37	62.99 ± 0.34	62.96 ± 0.28
39	63.53 ± 0.40	63.55 ± 0.26
41	65.34 ± 0.60	65.27 ± 0.31
43	66.16 ± 0.62	66.59 ± 0.39
45	67.84 ± 0.51	67.39 ± 0.65
47	68.34 ± 0.58	68.22 ± 0.52
49	69.49 ± 0.41	69.19 ± 0.27
51	70.19 ± 0.29	70.22 ± 0.38
53	70.61 ± 0.35	70.91 ± 0.45
55	71.24 ± 0.35	71.41 ± 0.32
57	71.47 ± 0.26	71.36 ± 0.38
59	72.49 ± 0.36	72.19 ± 0.33
61	72.69 ± 0.50	71.95 ± 0.33
63	71.33 ± 0.12	71.08 ± 0.17
65	71.72 ± 0.35	71.97 ± 0.29

[1] Values are the mean ± SEM of 9 replicate pens of 38 hens per treatment.

Table 3.16. Specific gravity of eggs from broiler breeder hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 21 (onset of photostimulation) through 65 wk of age (Experiment 3)[1].

	Dietary treatment	
Age	Control	AZ
wk		
30	1.081 ± 0.0003	1.081 ± 0.0003
35	1.080 ± 0.0004	1.079 ± 0.0004
39	1.081 ± 0.0005	1.080 ± 0.0004
44	1.079 ± 0.0008	1.078 ± 0.0010
48	1.078 ± 0.0005	1.077 ± 0.0006
53	1.076 ± 0.0004	1.073 ± 0.0003*
58	1.072 ± 0.0005	1.071 ± 0.0004
65	1.076 ± 0.0005	1.076 ± 0.0006

[1] Values are the mean ± SEM of 9 replicate pens of 38 hens per treatment.

Table 3.17. Fertility and hatchability of eggs produced through 65 wk of age by broiler breeder hens fed a control diet or this diet supplemented with 0.25% AZOMITE (AZ) from 21 (onset of photostimulation) through 65 wk of age (Experiment 3)[1].

Item	Control	AZ
	%	
Fertility	80.97 ± 1.66	84.27 ± 1.80
Hatchability[2]	75.35 ± 1.75	77.69 ± 1.90
Hatch of fertile	93.01 ± 0.45	92.15 ± 0.35
Early dead embryos[3,4]	2.21 ± 0.15	1.91 ± 0.25
Late dead embryos[3,4]	1.87 ± 0.24	1.73 ± 0.22
In-shell[3,4]	1.55 ± 0.10	2.94 ± 0.17*

[1] Values are the mean ± SEM of 9 replicate pens of 38 hens per treatment. Ninety eggs from each replicate pen were incubated and hatched at 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56 and 59 wk of age. *AZ value for a given parameter differs from control value, ($P < 0.05$).

[2] Hatch of eggs set

[3] Embryo mortality was classified as early dead (less than 14 d) or late dead (15-21 d of incubation) embryos. In shell included both live and dead –in-shell at the time of hatch.

[4] Calculated as a percentage of fertile eggs.

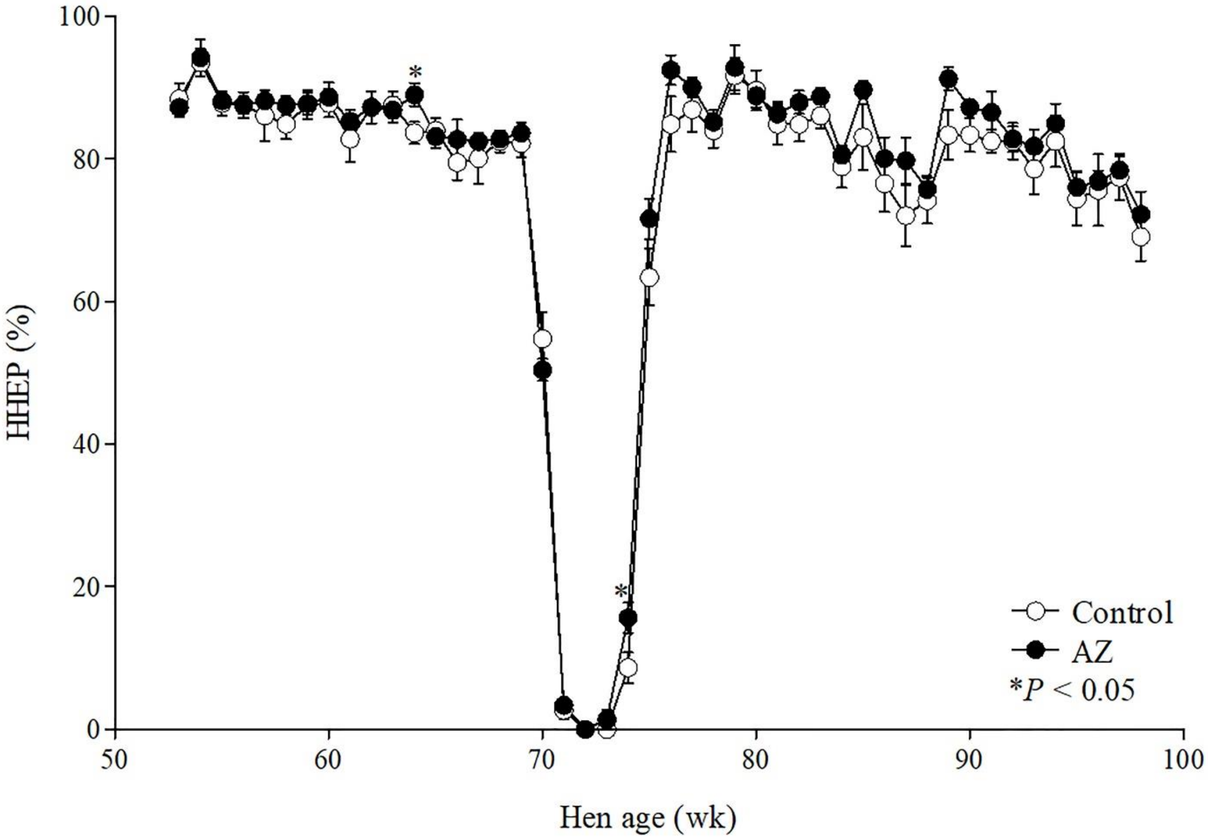

Figure 3.1: Weekly hen housed egg production (HHEP) of laying hens fed a control diet or this diet supplemented with 0.25% Azomite from 54 to 98 weeks of age. Values are means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens. Note hens were fed a molting diet from 71 through 72 weeks of age.

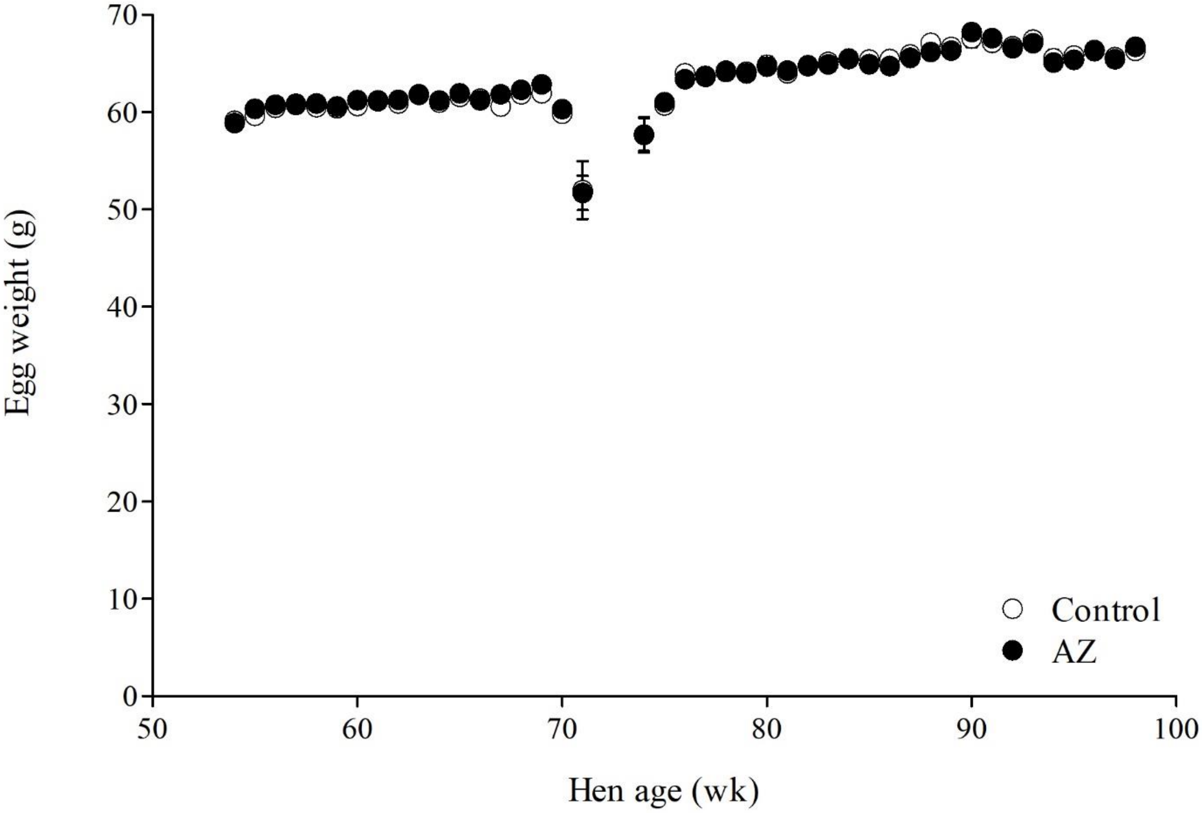

Figure 3.2: Egg weight of eggs produced from laying hens fed a control diet or this diet supplemented with 0.25% Azomite. Values are means ± SEM of 6 replicate groups of hens with each group containing 8 individually caged hens. Note hens were fed a molting diet from 71 through 72 weeks of age.

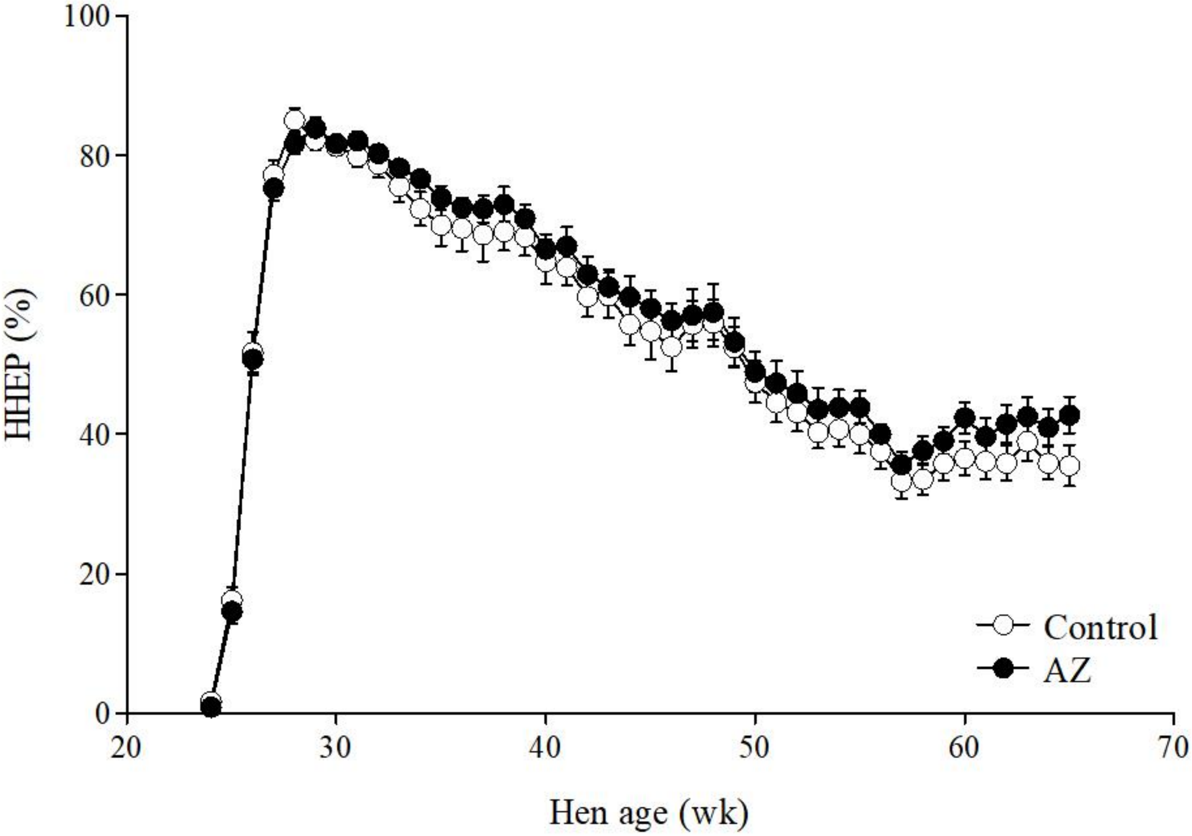

Figure 3.3: Weekly hen housed egg production (HHEP) of broiler breeder hens fed a control diet or this diet supplemented with 0.25% Azomite from 21 (onset of photostimulation) through 65 weeks of age. Values are means ± SEM of 9 replicate pens of 38 hens per treatment.

CHAPTER 4

EGG PRODUCTION IS IMPROVED IN HEAT STRESSED LAYING HENS FED A DIET CONTAINING SELENOMETHIONINE HYDROXY ANALOGUE RATHER THAN SODIUM SELENITE [1]

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[1] Juzaitis-Boelter, C. P., A. J. Davis, M. De Marco, J. Brackenridge, and R. B. Shirley. To be submitted to *Poultry Science*.

ABSTRACT

Selenium is an essential nutrient that is often added to poultry diets using inorganic sources such as sodium selenite. However, research suggests that using organic sources of selenium may enhance selenium absorption and retention. The function of selenium has largely been attributed to its presence in selenoproteins whose functions include antioxidant defense, thyroid hormone metabolism and immune and stress responses. Therefore, the objective of this experiment was to determine if heat stressed laying hens fed diets supplemented with an organic selenium source [Selisseo®, (selenomethionine hydroxy analogue) or Sel-Plex®,(a seleno-yeast product) would produce more eggs than heat stressed hens fed a diet containing inorganic selenium (sodium selenite). HyLine WD-36 laying hens were fed a layer diet supplemented with equal levels of selenium derived from either sodium selenite, Sel-Plex® or Selisseo® from 42 through 71 weeks of age. Each dietary treatment was provided to 120 cages with 2 hens per cage. Throughout the experiment, the average ambient hen house temperature during the day and night was greater than 30.3° C, and 29.7° C, respectively. Hen body weights and feed consumption were not different throughout the experiment between the dietary treatments. Marketable egg production was significantly ($P < 0.05$) greater (5 eggs per bird) for the hens fed Selisseo® relative to those fed sodium selenite, while marketable egg production in hens fed Sel-Plex® did not differ from the other 2 treatments. Egg weight, Haugh units and yolk color did not differ between the treatments, but the selenium content of eggs was greater ($P < 0.000$) in hens fed Selisseo® compared to the other 2 treatments. The results indicate that feeding Selisseo® can lessen the detrimental effects of heat stress on laying hens.

Key Words: organic selenium, inorganic selenium, egg quality

INTRODUCTION

Modern commercial layers are bred for high reproductive efficiency. In order to satisfy the physiological requirements of abundant egg production, it is imperative that nutrient absorption is optimized. Previous research has shown that high ambient temperatures reduce nutrient digestibility in broilers (Seven and Seven, 2008) as well as feed efficiency and egg production in quail (Sahin et al., 2008). In laying hens, heat stress also decreases egg production, egg weight and shell quality (Kirunda et al., 2001; Emery et al., 1984; Mashalay et al., 2004). Environmental conditions that elicit heat stress insult commercial poultry productivity at the molecular level via the overproduction of free radicals such as superoxide and, subsequently, give rise to oxidative damage (Mashalay et al., 2004; Mujahid et al., 2005; Mujahid et al., 2007). As reviewed by Brigelius-Flohé, (1999) and Surai, (2002), selenium has been established as a trace mineral of profound importance with respect to antioxidant status because the conversion of membrane-impairing hydrogen peroxides and hydroperoxides to non-reactive products relies on selenium-dependent glutathione peroxidases. Accordingly, the antioxidant defenses of selenium are most significant, and have been shown to be more advantageous, when poultry are subjected to stressful conditions (Pappas et al., 2006).

As extensively reviewed by Surai et al., (2018), when the primary tissue storage form of selenium, selenomethionine (SeMet), is provided in poultry diets, the efficacy of selenium transfer to the muscles is increased, resulting in enhanced selenium availability for selenoprotein synthesis. Evidence that the dietary form of selenium acts as a pivotal determinant of its bioavailability has mostly come to light over the last few decades (Briens et al., 2013). Although inorganic sources of selenium such as sodium selenite have traditionally served as staples in the selenium supplementation of poultry diets, there are a number of disadvantages associated with inorganic forms of selenium, such as low selenium retention rates and relatively high toxicity levels, which may correspond with observations that selenite has a pro-oxidant effect (Yan and Spallholz, 1993). Research indicates that SeMet is retained more efficiently than sodium selenite and thereby enhances antioxidant capacity (Sahin et al., 2008; Xiao et al., 2016). Similarly, observations of increased breast muscle selenium concentrations and selenium deposition in the albumen and yolk of broiler breeders supplemented with SeMet compared to sodium selenite supplementation provides further evidence for enhanced selenium retention in poultry when SeMet is the dietary source utilized (Wang et al., 2011).

In the last decade, selenomethionine hydroxy analogue (R,S-2-hydroxy-4-methylselenobutanoic acid; HMSeBA), has become a common organic source of selenium used in animal diets. Previous research with an HMSeBA product known as Selisseo® (Adisseo, Alpharetta, GA) indicates that breast muscle selenium deposition in broilers (Briens et al., 2013) and tissue and egg selenium deposition in laying hens (Jlali et al., 2013) is enhanced with this product when compared to other organic and inorganic selenium sources. Selenium deficiencies have been shown to concomitantly suppress selenoprotein synthesis and immune function (Khoso et al., 2015). Thus, the upregulation of selenoprotein production may serve as a mechanism by which birds mitigate the physiological effects of environmental stressors, such as heat stress. Therefore, enhanced retention of selenium through dietary supplementation with organic selenium sources compared to inorganic sources could be advantageous to heat stressed laying hens. The goal of the current research was to determine if heat stressed laying hens fed diets supplemented an organic selenium source [Selisseo® or Sel-Plex® (a seleno-yeast product, Alltech, Lexington, KY)], produced more eggs than hens fed an inorganic source of selenium (sodium selenite).

MATERIALS AND METHODS

During a 4 wk pre-experimental period egg production was monitored in 768, 38-wk-old, Hy Line W-36 White Leghorn laying hens fed a standard laying hen diet (Table 4.1). The hens had been raised from day of age under standard protocols at the University of Georgia. The hens were housed in 4 rows of a single tiered suspended cage system, with each cage being 45.75 cm high, 25.4 cm wide and 40.64 cm deep. Each cage housed 2 hens, had a sloped floor for egg displacement, and had an egg holding area underneath the individual trough feeder that attached to the front of each cage. Each cage was also equipped with a nipple drinker. The facility housing the hens had 2 thermostatically controlled gas-fired heaters, four, 61 cm circulation fans, and 4 ventilation fans (three, 43 cm fans and one, 91 cm fan). The facility was not equipped with any cooling capacity beyond air movement. For the duration of the study, the daily lighting schedule was lights on at 07:00 hours and off at 21:00 hours. Eggs were collected daily. All experimental animal procedures were approved by the University of Georgia Animal Care and Use Committee.

At the end of the third wk of the pre-experimental period all the hens were weighed and egg production per cage was determined. The 360 cages with hens producing the most eggs were selected and distributed into 3 treatment groups such that each treatment group had 3 blocks of 10 cages in each of the 4 rows of suspended cages giving each treatment group 12 blocks of 10 cages or a total of 120 cages with each cage containing 2 hens. With this selection process, egg production and BW did not differ among treatments or individual blocks.

The hens were given a 1-wk period to re-assimilate, to confirm that egg production and BW still did not differ between treatments and to confirm egg weight and specific gravity did not differ between treatments, before commencing the experiment when the birds started their 42 wk of age. The experiment started in mid-June which naturally provided heat stress conditions in the Southeastern United States, but to ensure constant elevated temperatures the thermostat for the heaters was set at 28.8° C in the facility. Hobo UX-100-023A data loggers (Onset Computer Corporation, Bourne MA) were used to continuously record temperature and humidity every 5 minutes for the duration of the experiment.

From 42 through 71 weeks of age, the hens were fed a laying hen diet supplemented with 0.3 ppm sodium selenite, Sel-Plex® or Selisseo® (Table 4.1). Given the decrease in egg production and increase in body weight gain, along with the potential decrease in shell quality in aged hens, when the hens reached 63 wk of age the energy and digestible amino acid content of the laying hen diet was decreased and the calcium content was increased (Table 4.1). All bulk dietary ingredients were analyzed for nutrient composition (Midwest Laboratories, Omaha, NE) and the 3 treatment diets were mixed every 3 weeks throughout the experiment for a total of 10 batches for the 30 wk experiment. The selenium content of the selenium sources and completed diets was confirmed by Eurofins Scientific Inc (Des Moines, IA). The total selenium content for each of the 3 diets in each of the 10 batches was 0.40, 0.38, 0.36, 0.36, 0.42, 0.42, 0.36, 0.39, 0.39 and 0.39 ppm, respectively.

During the experiment, all hens were individually weighed every 3 wk, and total feed consumption per cage was also determined every 3 wk. Eggs were manually collected once per d in the late afternoon, and at the time of egg collection, all eggs were classified as normal (marketable eggs), cracked, double-yolked, misshapen, small, membrane, or dirty. Marketable and nonmarketable eggs were weighed daily on a per block basis. Hen-housed and hen-day egg production were calculated weekly from daily egg counts. At the end of every 3 wk experimental interval, 2 d-worth of egg production was saved to ensure that 2 individual eggs from each cage were available for the measurement of specific gravity, egg weight, Haugh unit, egg yolk color, albumen weight and yolk weight. Specific gravities were determined following the procedure of Phillips and Williams (1944). Following specific gravity measurements, the 2 representative eggs from each cage were stored at room temperature for 2 wk prior to egg component analysis. Haugh unit measurements were completed on both eggs from each cage. One egg from each cage was used to measure egg yolk color, albumen weight, and yolk weight. Egg yolk color was assessed using a Minolta colorimeter (Konica Minolta, Tokyo, Japan) to measure L* measure (lightness), a* (redness), and b* (yellowness). The tip of the colorimetric measuring head was placed flat against the surface of each individual yolk for the measurements. Albumen and egg yolk components from 3 randomly selected eggs from each block were pooled and stored at -80° C for Se content analysis.

At the conclusion of the experiment, the 2 cages from each block having egg production closest to the mean egg production of the block for the preceding 3 weeks were selected. Blood was collected from each of the hens in these 2 cages to obtain serum samples from each bird. Serum samples were frozen at -80° C. The hens were then killed and the liver was visually scored and liver color was assessed with the Minolta colorimeter. Livers were then removed and frozen at -80° C. The liver score was based on a visual and tissue assessment with scores ranging from 1 to 10. A score of 1 was given to a liver that had a rich brown red color for the entire surface, had no visual lesions or hemorrhages and whose tissue remained intact as handled. A score of 10 was given to a liver that had a light tan color for the entire surface indicating complete infiltration by lipid, had widespread lesions and/or hemorrhages and whose tissue completely disintegrated when handled.

Selenium content

Albumen and egg yolk samples from 8 out of the 12 replicate blocks for each dietary treatment were randomly selected for Se analysis when the hens were 41 (pre-experiment), 47, 53, 59, 65, and 71 weeks of age. Prior to analysis, egg yolk and albumen samples for each selected block were thawed on ice, combined, and homogenized respective of block in preparation for freeze drying. Samples were weighed, freeze-dried using a VirTis Genesis 25L Pilot Lyophilizer (SP Scientific, Stone Ridge, NY) over a 33-h period and subsequently weighed again to obtain post freeze-dried weights. Freeze-dried samples were analyzed for Se content by the Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI).

Thiobarbituric acid reactive substances (TBARS) assay

The measurement of TBARS is a well-established method for screening and monitoring lipid peroxidation and measures the malondialdehyde (MDA) content in the assay reaction. Both liver and serum samples collected at the end of the experiment were used for TBARS analyses using the Cayman Chemical TBARS (TCA Method) Assay Kit (Ann Arbor, MI) following the manufacturer’s protocols. A VICTOR® Nivo multimode microplate reader (PerkinElmer, Hopkinton, MA) was used for the colorometric analysis of the samples.

Statistical analysis

Data were subjected to ANOVA according to the General Linear Model (GLM) with dietary treatment as the factor in the statistical model. Tukey’s multiple-comparison procedure was used to detect significant differences among individual dietary treatments (Neter et al., 1990). Differences were considered significant when $P < 0.05$. All statistical procedures were completed with the Minitab statistical software package (Release 16, State College, PA).

RESULTS

Because this experiment commenced in mid-June in the Southeastern United States, the facility housing the hens had high ambient temperatures and humidity (Table 4.2). As the experiment continued in to fall and winter the high average house temperature was maintained with the gas-fired furnaces, but the humidity decreased (Table 4.2). Additionally, as the furnaces provided more of the heat to provide the elevated temperatures the differential between the mean day and night temperatures tended to decrease.

Body weight (Table 4.3) and feed consumption (Table 4.4) of the hens did not differ between the treatments throughout the experiment. Weekly hen housed egg production and cumulative hen housed egg production on a percent basis (Table 4.5) typically did not differ between treatments, but for some individual weeks especially as the experiment progressed, there were weeks in which the hens supplemented with Selisseo® did have significantly ($P < 0.05$) greater egg production than the hens supplemented with sodium selenite. In addition, numerically the hens fed the diet containing Selisseo® had a consistent 1.5 to 2% greater cumulative total hen housed egg production than the hens fed the diet containing sodium selenite (Table 4.5). This difference led to a significant ($P < 0.05$) increase in the total number of eggs produced and total number of marketable eggs produced by the hens fed the diet supplemented with Selisseo® compared to those fed the diet supplemented with sodium selenite (Table 4.6). Total mortality was equal between the treatments with 6 hens in each treatment dying.

Egg weights were determined daily on a block basis to calculate a weekly mean egg weight per treatment (Table 4.7) and measured on cage basis every 3 wk (Table 4.8) and in both cases egg weight did not differ between the sodium selenite treatment when compared to either of the organic selenium treatments. The specific gravity of eggs did not vary between the 3 dietary treatments except at 62 wk of age when the eggs from the Sel-Plex-fed hens had a decreased specific gravity relative to the eggs produced by hens fed the sodium selenite diet (Table 4.9). Throughout the entire experiment, Haugh unit determinations did not differ in the eggs produced from hens fed the 3 different selenium sources (Table 4.10). When examining individual egg component weights (Table 4.11), yolk and shell weight did not differ between the dietary treatments and albumen weight only differed at 1 time point out of 10 and that difference was only on a gram basis and not as a % of the whole egg. Yolk color also did not differ in the eggs produced by the hens fed the different sources of dietary selenium (Table 4.12). Finally, while the selenium content of combined albumen and yolk samples from eggs produced by the hens from each of the three individual dietary treatments was equal at the start of the experiment (Table 4.13), as the experiment progressed, the selenium content was greater in samples obtained from eggs produced by the hens fed the Selisseo® treatment than the other 2 selenium dietary treatments.

Not surprisingly, given that body weight (Table 4.3), feed consumption (Table 4.4) and egg weight (Tables 4.7-4.8) did not differ between the treatments, while total egg production was greater in the Selisseo®-fed hens compared to the sodium selenite-fed hens (Table 4.6), the feed efficiency for egg production was improved in the Selisseo® treatment relative to the sodium selenite treatment on a cumulative basis from week 57 of age to the end of the experiment (Table 4.14).

At the end of the experiment, the liver score and color of the liver did not differ among the 3 dietary treatments (Table 4.15) indicating at the gross level that fat content of the liver did not vary greatly. In addition, based on TBARS analyses the amount of malondialdehyde derived from the decomposition of unstable lipid peroxides did not differ in serum and liver samples from hens from the 3 dietary treatments (Table 4.16).

DISCUSSION

Based on increased egg production, the current research indicates that using Selisseo® as the source for dietary selenium supplementation can alleviate some effects of heat stress in laying hens compared to using Sel-Plex®, another source of organic selenium, or sodium selenite, an inorganic source of selenium. Additionally, using Selisseo® as the dietary source of selenium in laying hen diets during heat stress increased selenium deposition in eggs compared to using Sel-Plex® or sodium selenite. Jlali et al., (2013) reported that egg selenium concentrations in hens fed organic sources of selenium (Selisseo® and Sel-Plex®) were greater than those fed diets supplemented with sodium selenite. But, notably, laying hens supplemented with Selisseo® had significantly greater selenium deposition in both their eggs as well as muscles compared to hens fed diets supplemented with Sel-Plex® (Jlali et al., 2013). As reviewed by Surai et al., (2018), and as reported by Payne et al., (2005), enhanced selenium transfer to the egg can equate to improvements in egg quality, as indicated by improved Haugh unit measurements. However, it is likely that improvements in egg quality were not observed in the current research as the hens were subjected to heat stress as opposed to thermoneutral environments utilized in previous research. Previously, Abd El-Hack et al., (2017) reported that feeding heat stressed Bovans Brown laying hens diets with increased dietary levels of sodium selenite did not improve egg production compared to control hens fed a diet with a standard level of sodium selenite. Taken together the current research and the previous research suggests that organic sources of dietary selenium are more efficacious in laying hens than inorganic sources and that Selisseo® may be more effective than Sel-Plex®.

Other previous non-laying hen research has also indicated that supplementing poultry diets with organic sources of selenium may be more beneficial than using inorganic sources. Liao et al., (2012) found that broiler diets supplemented with a selenium-enriched yeast product had significantly greater selenium concentrations in the liver and breast muscle compared to broilers supplemented with sodium selenite. Mahmoud and Edens, (2003) observed that while broilers fed Sel-Plex® had significantly greater whole blood glutathione peroxidase activity relative to broilers fed sodium selenite, the effects upon glutathione peroxidase activity were even further enhanced when the birds were subjected to heat stress. Previous research in broiler breeders have also provided evidence that selenium deposition into the liver, kidney, pancreas, and breast muscle was enhanced when birds were supplemented with organic sources of selenium (Sel-Plex® and selenomethionine) compared to birds fed diets supplemented with sodium selenite (Yuan et al., 2011). In addition, Yuan et al., (2011) found that selenium concentrations in the yolk and albumen of selenomethionine-supplemented birds were significantly greater than in those supplemented with Sel-Plex®, which mirrors the observations in the current research.

As reviewed by Surai et al., (2018), deviations in the efficacy of dietary selenium sources is owed to either the absence of, or variations in content of, selenomethionine. Vertebrate animals cannot synthesize selenomethionine. Although inorganic selenium sources such as selenite and selenate can be used for the formation of selenocysteine, which is the form used in selenoprotein biosynthesis, only selenomethionine can randomly be incorporated into body proteins by replacing methionine, which provides a non-specific stored pool of selenium that is available for release and utilization with normal metabolism. Selenomethionine products produced by yeast are variable in composition due to the variability of dietary selenomethionine content associated with selenium-enriched yeasts. Indeed, as reviewed by Rayman, (2004), the selenomethionine level in selenium-enriched yeasts can vary from 60-70% - a consequence of batch-to-batch variability. In contrast, Selisseo® is a nearly pure product of selenomethionine hydroxyanalogue (purity > 99% HMSeBA), which can readily be converted by animals to selenomethionine and may explain why some studies have shown that the relative bioavailability for total selenium increased in Selisseo®-supplemented broilers compared to Sel-Plex®-supplemented birds (Briens et al., 2013). In a review by Surai and Fisinin, (2016), the authors discussed how the variation in selenomethionine content of selenium-enriched yeasts impacts the ability to guarantee selenomethionine content during production and thereby hinders its value as an organic source of selenium. These observations may contribute to the differences in efficacy of the 2 organic sources of selenium used in the current research.

Heat stress in broilers induces oxidative stress (Altan et al., 2003; Lin et al., 2000; 2006; Mahmoud and Edens, 2003), and given the increased egg production in hens fed Selisseo® it could be hypothesized that these hens might be suffering from less heat stress-induced oxidative stress. However, the amount of malondialdehyde derived from the decomposition of unstable lipid peroxides did not differ in serum and liver samples from hens fed the 3 different dietary sources of selenium. Petrovič et al., (2006) also reported that plasma malondialdehyde content did not differ in laying hens in which the dietary selenium supplementation source was either sodium selenite or Sel-Plex®. In the current research, although Selisseo supplementation did not reduce plasma and liver malondialdehyde production, it is important to note that this is only one measure of oxidative stress. Similar future research could examine oxidative stress more conclusively by measuring the activity of glutathione peroxidases and thioredoxine reductases. Mahmoud and Edens, (2003) and Zhang et al., (2014) both detected significant differences in glutathione peroxidase activity in broilers between inorganic and organic selenium sources, in both heat-stressed and thermoneutral conditions, respectively. Additionally, in highly productive laying hens, given the high level of hepatic production of yolk lipids and their blood transport to the ovary, the ability to detect differences in lipid peroxidation may not be possible. In the current research, while the coefficient of variation in malondialdehyde concentrations in liver and serum samples between duplicate samples was less than 5%, it was greater than 75% and 25% for hepatic and serum samples within each treatment, respectively. Thus, completing the TBARS assay in tissues not effected by yolk lipids such as muscle tissue could be more fruitful in laying hen research.

In summary, feeding heat stressed laying hens diets in which the supplemented selenium was provided by Selisseo® rather than Sel-Plex® or sodium selenite results in increased egg production, improved feed to egg production efficiency and greater selenium deposition in eggs. Further research will be necessary to determine the mechanism by which Selisseo® lessens the impact of heat stress on laying hens.

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Table 4.1. Composition of the laying hen diets.

Ingredient	Pre-experimental[1]	Experimental diet at 42 weeks of age[2]			Experimental diet at 63 weeks of age[3]		
		Sodium Selenite	Sel-Plex	Selisseo	Sodium Selenite	Sel-Plex	Selisseo
				%			
Corn	52.092	51.847	51.789	51.844	56.408	56.349	56.329
Soybean meal (48%CP)	18.572	19.128	19.128	19.128	13.896	13.896	13.896
DDGS (corn)	12.000	12.000	12.000	12.000	12.000	12.000	12.000
Course limestone	4.7300	4.716	4.734	4.717	6.165	6.188	6.196
Fine limestone	4.7300	4.716	4.734	4.717	3.853	3.868	3.873
MBM	3.879	3.452	3.452	3.452	4.000	4.000	4.000
Soybean oil	2.811	2.877	2.897	2.878	2.087	2.108	2.115
Salt	0.303	0.313	0.313	0.313	0.348	0.348	0.348
Sodium carbonate	0.088	0.088	0.088	0.088	0.088	0.088	0.088
L-Lysine, HCl 78.8%	0.038	0.034	0.034	0.034	0.034	0.034	0.034
DL- Methionine 99%	0.125	0.126	0.126	0.126	0.104	0.104	0.104
Tribasic copper chloride	0.000	0.015	0.015	0.015	0.015	0.015	0.015
Dicalcium phosphate	0.000	0.000	0.000	0.000	0.252	0.252	0.252
Choline chloride 60%	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Phytase[4]	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Vitamin mix[5]	0.567	0.567	0.567	0.567	0.567	0.567	0.567
Mineral mix[6]	0.094	0.000	0.000	0.000	0.000	0.000	0.000
Se free mineral mix[7]	0.000	0.100	0.100	0.100	0.100	0.100	0.100
Sodium Selenite[8]	0.000	0.050	0.000	0.000	0.050	0.000	0.000
Sel-Plex[9]	0.000	0.000	0.050	0.000	0.000	0.050	0.000
Selisseo mix[10]	0.000	0.000	0.000	0.050	0.000	0.000	0.050
Calculated analysis							
AME (kcal/kg)	2850	2850	2850	2850	2790	2790	2790
Crude protein (%)	18.176	18.151	18.147	18.151	16.942	16.938	16.937
Calcium (%)	4.000	4.000	4.000	4.000	4.200	4.200	4.200
Available phosphorus (%)	0.373	0.373	0.373	0.373	0.411	0.411	0.411
Digestible TSAA[11] (%)	0.630	0.630	0.630	0.630	0.580	0.580	0.580

Digestible lysine (%)	0.750	0.750	0.750	0.750	0.690	0.690	0.690
Digestible threonine (%)	0.562	0.563	0.563	0.563	0.512	0.512	0.512

[1] Preexperimental diet was fed from 38 through 41 weeks of age.

[2] These diets were fed from 42 through 44 weeks of age. The diets fed from 45 through 62 weeks of age were similar in composition and had exactly the same calculated content of AME, calcium, available phosphorus, digestible total sulfur amino acids, lysine and threonine as the diets fed from 42-44 weeks of age.

[3] These diets were fed from 63 through 65 weeks of age. The diets fed from 66 through 71 weeks of age were similar in composition and had exactly the same calculated content of AME, calcium, available phosphorus, digestible total sulfur amino acids, lysine and threonine as the diets fed from 63-65 weeks of age.

[4] Quantum blue phytase (5,000 FTU/g, AB Vista Plantation, FL)

[5] Vitamin premix (DSM custom vitamin premix. DSM Nutritional Products, Inc. Parsippany, NJ) provides the following per kilogram of diet: vitamin A, 5,510 IU; vitamin D_3, 1,100 IU; vitamin E, 11 IU; vitamin B_{12}, 0.001mg; riboflavin, 4.4 mg; niacin, 44 mg; d-panthotenic acid, 12 mg; choline chloride, 220 mg; menadione sodium bisulfate, 3.34 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin mononitrate, 2.2 mg; d-biotin, 0.11 mg; and ethoxyquin, 125 mg.

[6] Trace mineral premix (Southeastern Minerals custom trace mineral mix. Southeastern Minerals Inc. Bainbridge, GA) provides the following in milligrams per kilogram of diet: manganese, 60; zinc, 50; iron, 30; copper, 5; iodine, 1.5; selenium, 0.5. Trace mineral forms were manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, calcium iodate, and sodium selenite.

[7] Selenium free trace mineral premix contained 28.17% zinc sulfate monohydrate, 37.50% Manganese sulfate monohydrate, 3.97% copper sulfate pentahydrate, 10% ferrous sulfate monohydrate, 0.22% calcium iodate, 3.08% mineral oil, and 17.07% rice hulls and provides the following milligrams per kilogram of diet zinc, 100; manganese, 120, copper, 10; iron, 30; and iodine, 1.4.

[8] Phibro 0.06% Selenium Premix NC, (Prince Agri Products Inc. Quincey, IL)

[9] Nutra Blend Sel-Plex 600 (Nutra Blend LLC, Neosho, MO).

[10] Selisseo, (Adisseo USA Inc., Alpharetta, GA) premix contained 0.06% Selenium and consisted of (95.57% wheat middlings, 3% Selisseo (2% Se) and 1.43% mineral oil.

[11] TSAA = Total sulfur amino acids.

Table 4.2. Average house temperature and relative humidity during each experimental period[1].

Hen age (wk)	Average temperature and relative humidity from 9 am to 9 pm		Average temperature and relative humidity from 9 pm to 9 am		NOAA average daily outside temperature and relative humidity[2]	
	°F	%	°F	%	°F	%
42-44	87.69	60.97	85.60	64.04	79.71	70.70
45-47	87.56	59.33	85.69	63.88	79.76	69.31
48-50	88.04	62.43	85.71	66.79	80.29	72.06
51-53	87.24	58.96	85.76	60.85	77.62	68.74
54-56	88.51	51.91	86.85	54.81	77.14	66.83
57-59	88.31	46.67	87.63	47.34	68.24	71.45
60-62	86.84	57.62	86.56	56.58	57.90	72.04
63-65	86.72	38.62	85.84	38.78	51.90	62.78
66-68	87.25	50.30	87.01	47.29	47.19	65.11
69-71	87.44	49.65	86.91	48.41	53.67	68.62

[1] Values are means from 4 HOBO (Onset Computer Corporation, Bourne, MA) data loggers distributed throughout the experimental area. Temperature and relative humidity were captured every 5 minutes.

[2] National Oceanic and Atmosphere Administration (NOAA) station at Ben Epps Airport, (Athens, GA).

Table 4.3. Body weight of heat stressed hens fed different dietary selenium sources[1].

Hen age	Dietary treatment		
	Sodium selenite	Sel-Plex	Selisseo
wk		g	
42	1835 ± 10	1832 ± 11	1830 ± 11
45	1881 ± 10	1875 ± 11	1872 ± 11
48	1943 ± 12	1926 ± 13	1925 ± 13
51	1980 ± 12	1961 ± 13	1967 ± 13
54	2035 ± 13	2008 ± 13	2003 ± 13
57	2057 ± 13	2028 ± 13	2020 ± 14
60	2082 ± 13	2054 ± 14	2049 ± 14
63	2099 ± 14	2072 ± 14	2069 ± 14
66	2042 ± 14	2023 ± 13	2014 ± 14
69	2073 ± 17	2067 ± 14	2052 ± 14
72	2087 ± 15	2068 ± 14	2058 ± 14

[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage.

Table 4.4. Feed consumption of heat stressed hens fed different dietary selenium sources[1].

Hen age	Dietary treatment		
	Sodium selenite	Sel-Plex	Selisseo
wk		g/day	
42-44	112.81 ± 0.55	112.12 ± 0.52	111.46 ± 0.41
45-47	120.56 ± 0.93	120.34 ± 0.94	120.19 ± 0.87
48-50	117.73 ± 0.73	117.65 ± 0.84	117.28 ± 0.68
51-53	115.95 ± 0.81	115.37 ± 0.85	114.92 ± 0.73
54-56	115.22 ± 0.81	115.48 ± 0.93	115.21 ± 0.92
57-59	114.50 ± 0.83	114.77 ± 0.96	113.37 ± 0.78
60-62	116.78 ± 0.89	117.22 ± 0.83	116.72 ± 0.88
63-65	113.46 ± 0.85	115.78 ± 0.94	115.26 ± 0.89
66-68	118.76 ± 0.89	120.28 ± 0.95	119.78 ± 0.87
69-71	121.56 ± 0.81	121.58 ± 0.90	122.46 ± 0.83

[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage.

Table 4.5. Weekly hen day egg production and hen housed total egg production for heat stressed hens fed different dietary selenium sources[1].

Hen Age	Weekly hen day egg production			Cumulative total hen housed egg production		
	Sodium selenite	Sel-Plex	Selisseo	Sodium selenite	Sel-Plex	Selisseo
wk				%		
42	92.80 ± 0.80	93.15 ± 0.57	92.80 ± 0.65	92.80 ± 0.80	93.15 ± 0.57	92.80 ± 0.65
43	92.62 ± 0.82	94.46 ± 0.58	94.40 ± 0.59	92.71 ± 0.66	93.81 ± 0.45	93.60 ± 0.51
44	91.73 ± 0.82	92.20 ± 0.67	92.38 ± 0.58	92.38 ± 0.63	93.27 ± 0.44	93.19 ± 0.44
45	90.24 ± 0.79	91.61 ± 0.74	91.31 ± 0.62	91.84 ± 0.57	92.86 ± 0.45	92.72 ± 0.39
46	89.52 ± 0.85	90.42 ± 0.67	91.49 ± 0.72	91.38 ± 0.53	92.37 ± 0.43	92.48 ± 0.39
47	88.21 ± 0.86	87.92 ± 0.98	90.00 ± 0.74	90.85 ± 0.49	91.63 ± 0.46	92.06 ± 0.38
48	90.12 ± 0.81^{ab}	88.10 ± 0.96^{b}	91.01 ± 0.64^{a}	90.75 ± 0.46	91.12 ± 0.47	91.91 ± 0.37
49	89.46 ± 0.88	89.52 ± 0.86	91.31 ± 0.62	90.59 ± 0.46	90.92 ± 0.48	91.84 ± 0.35
50	88.51 ± 1.02	89.40 ± 0.96	91.31 ± 0.65	90.36 ± 0.46	90.75 ± 0.49	91.78 ± 0.65
51	86.31 ± 1.16	86.67 ± 0.93	88.21 ± 0.79	89.95 ± 0.48	90.35 ± 0.51	91.42 ± 0.35
52	87.02 ± 1.05	87.80 ± 0.86	89.82 ± 0.81	89.69 ± 0.50^{b}	90.11 ± 0.50^{ab}	91.28 ± 0.36^{a}
53	88.04 ± 0.89	88.69 ± 0.81	88.15 ± 0.81	89.55 ± 0.50	90.00 ± 0.50	91.02 ± 0.37
54	85.11 ± 1.03	85.41 ± 1.11	86.44 ± 0.92	89.15 ± 0.52	89.59 ± 0.52	90.61 ± 0.39
55	84.57 ± 1.19	86.38 ± 1.11	86.68 ± 1.00	88.78 ± 0.55	89.31 ± 0.54	90.28 ± 0.41
56	85.65 ± 1.10	85.05 ± 1.19	86.79 ± 0.87	88.52 ± 0.57	88.98 ± 0.57	90.00 ± 0.43

57	84.08 ± 1.06	85.71 ± 0.99	86.32 ± 0.86	88.15 ± 0.60	88.68 ± 0.59	89.68 ± 0.46
58	82.14 ± 1.16	85.35 ± 1.01	85.23 ± 1.02	87.72 ± 0.63	88.40 ± 0.61	89.33 ± 0.49
59	83.29 ± 1.16	85.29 ± 1.08	83.23 ± 1.11	87.40 ± 0.65	88.15 ± 0.64	88.92 ± 0.52
60	83.60 ± 1.03	85.41 ± 1.09	84.81 ± 1.07	87.12 ± 0.67	87.93 ± 0.67	88.63 ± 0.56
61	82.02 ± 1.22	84.08 ± 1.18	85.17 ± 1.10	86.80 ± 0.69	87.67 ± 0.70	88.38 ± 0.58
62	81.17 ± 1.32	82.99 ± 1.22	84.56 ± 0.87	86.47 ± 0.71	87.38 ± 0.72	88.13 ± 0.60
63	81.62 ± 1.23^{b}	83.09 ± 1.21^{b}	86.81 ± 0.87^{a}	86.16 ± 0.73	87.09 ± 0.75	87.97 ± 0.62
64	82.54 ± 1.11	83.58 ± 1.09	85.29 ± 0.99	85.91 ± 0.75	86.85 ± 0.77	87.76 ± 0.64
65	83.39 ± 1.12^{b}	83.03 ± 1.10^{b}	87.42 ± 0.95^{a}	85.72 ± 0.77	86.60 ± 0.79	87.66 ± 0.66
66	80.53 ± 1.23^{b}	82.36 ± 1.08^{ab}	84.31 ± 0.81^{a}	85.43 ± 0.79	86.35 ± 0.81	87.44 ± 0.68
67	82.97 ± 1.16	83.88 ± 1.22	85.35 ± 0.95	85.25 ± 0.81	86.17 ± 0.83	87.28 ± 0.69
68	81.38 ± 1.26	82.72 ± 1.18	83.15 ± 0.96	85.04 ± 0.83	85.97 ± 0.85	87.05 ± 0.71
69	80.04 ± 1.23	81.26 ± 1.10	83.52 ± 1.02	84.79 ± 0.85	85.73 ± 0.87	86.85 ± 0.73
70	79.55 ± 1.23	80.34 ± 1.33	82.30 ± 0.82	84.54 ± 0.86	85.47 ± 0.88	86.62 ± 0.74
71	77.41 ± 1.41^{b}	80.65 ± 1.27^{ab}	81.93 ± 1.12^{a}	84.23 ± 0.88	85.25 ± 0.90	86.39 ± 0.76

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[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage. $^{a-b}$Means with in a row for each parameter, differ $P < 0.05$.

Table 4.6. Egg production summary for heat stressed hens fed different selenium sources from 42 through 71 weeks of age[1].

Parameter	Sodium selenite	Sel-Plex	Selisseo
Total eggs produced per hen	179.24 ± 1.26^{b}	181.52 ± 1.25^{ab}	183.55 ± 0.99^{a}
Total marketable eggs produced per hen	177.04 ± 1.29^{b}	179.60 ± 1.25^{ab}	181.70 ± 1.00^{a}
Total cracked eggs produced per hen	0.45 ± 0.12	0.29 ± 0.06	0.22 ± 0.04
Total double yolk eggs produced per hen	0.11 ± 0.04	0.08 ± 0.02	0.10 ± 0.03
Total soft shelled eggs produced per hen	0.16 ± 0.04	0.10 ± 0.02	0.12 ± 0.04
Total dirty eggs produced per hen	1.31 ± 0.14	1.33 ± 0.25	1.28 ± 0.25
Total small eggs produced per hen	0.11 ± 0.03	0.10 ± 0.02	0.10 ± 0.04
Total misshaped eggs produced per hen	0.06 ± 0.02	0.03 ± 0.01	0.03 ± 0.01

[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage. $^{a-b}$Means with in a row for each parameter, differ $P < 0.05$.

Table 4.7. Egg weight of eggs produced by heat stressed hens fed different dietary selenium sources[1].

Hen Age	Dietary treatment		
	Sodium selenite	Sel-Plex	Selisseo
wk		g	
42	60.57 ± 0.23	60.84 ± 0.25	60.44 ± 0.29
43	60.15 ± 0.20	60.56 ± 0.23	59.92 ± 0.26
44	59.62 ± 0.16	60.17 ± 0.24	59.51 ± 0.29
45	60.68 ± 0.14	60.89 ± 0.33	60.35 ± 0.26
46	60.91 ± 0.17	61.20 ± 0.17	60.55 ± 0.31
47	61.16 ± 0.22	61.27 ± 0.28	60.75 ± 0.32
48	61.54 ± 0.22	61.89 ± 0.29	61.35 ± 0.33
49	61.80 ± 0.21	61.78 ± 0.26	61.55 ± 0.30
50	61.05 ± 0.16	61.19 ± 0.25	60.80 ± 0.34
51	61.84 ± 0.19	61.80 ± 0.27	61.49 ± 0.33
52	62.04 ± 0.23	62.42 ± 0.28	61.98 ± 0.22
53	62.24 ± 0.21	62.62 ± 0.25	61.80 ± 0.25
54	62.16 ± 0.24	62.59 ± 0.25	61.87 ± 0.26
55	62.50 ± 0.23	62.93 ± 0.23	62.49 ± 0.29
56	62.70 ± 0.18	63.06 ± 0.25	62.34 ± 0.26
57	62.51 ± 0.22	63.08 ± 0.28	62.31 ± 0.20
58	62.94 ± 0.21	63.25 ± 0.25	62.70 ± 0.17
59	63.03 ± 0.25	63.35 ± 0.23	62.84 ± 0.18
60	63.24 ± 0.21	63.46 ± 0.31	63.05 ± 0.22
61	63.52 ± 0.26	63.56 ± 0.33	63.40 ± 0.25
62	63.90 ± 0.25	63.98 ± 0.21	63.69 ± 0.30
63	63.95 ± 0.24	64.25 ± 0.27	63.82 ± 0.26
64	63.89 ± 0.21	64.15 ± 0.30	63.86 ± 0.32
65	63.66 ± 0.20	64.22 ± 0.28	63.68 ± 0.30

66	63.68 ± 0.24	63.91 ± 0.37	63.41 ± 0.29
67	63.74 ± 0.24	64.31 ± 0.31	63.36 ± 0.34
68	63.72 ± 0.24	64.27 ± 0.30	63.55 ± 0.25
69	63.83 ± 0.23	64.27 ± 0.36	63.52 ± 0.35
70	63.91 ± 0.27	64.20 ± 0.30	63.84 ± 0.30
71	64.15 ± 0.26	64.81 ± 0.33	64.43 ± 0.24

[1] Values are means ± SEM, n=12 blocks per treatment with each block consisting of 10 cages with each cage containing 2 hens. All marketable eggs were weighed per block on a daily basis. Daily values were then averaged to obtain weekly values.

Table 4.8. Egg weight of eggs produced by heat stressed hens fed different dietary selenium sources[1].

Hen age	Dietary treatment		
	Sodium selenite	Sel-Plex	Selisseo
wk		g	
44	60.10 ± 0.22	60.72 ± 0.24	60.18 ± 0.22
47	61.75 ± 0.24	61.82 ± 0.26	61.47 ± 0.27
50	61.09 ± 0.25	61.41 ± 0.27	61.18 ± 0.26
53	61.99 ± 0.27^{ab}	62.55 ± 0.28^{a}	61.59 ± 0.27^{b}
56	62.86 ± 0.27	62.94 ± 0.27	62.28 ± 0.26
59	63.23 ± 0.27	63.16 ± 0.29	62.49 ± 0.25
62	64.08 ± 0.30	64.33 ± 0.29	63.86 ± 0.31
65	63.77 ± 0.30	64.23 ± 0.27	63.55 ± 0.31
68	63.14 ± 0.28	63.98 ± 0.29	63.14 ± 0.30
71	64.08 ± 0.29	64.25 ± 0.31	64.26 ± 0.29
Overall	62.60 ± 0.21	62.90 ± 0.22	62.37 ± 0.21

[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage with the determination made on 2 eggs per cage collected at the end of each 3-week experimental period.

[a-b] Values within in a row with different superscripts differ, ($P < 0.05$).

Table 4.9. Specific gravity of eggs produced by heat stressed hens fed different dietary selenium sources[1].

Hen age	Dietary treatment		
	Sodium selenite	Sel-Plex	Selisseo
wk			
44	1.082 ± 0.000	1.081 ± 0.000	1.081 ± 0.000
47	1.081 ± 0.000	1.081 ± 0.000	1.081 ± 0.000
50	1.080 ± 0.000	1.080 ± 0.000	1.080 ± 0.000
53	1.079 ± 0.000	1.079 ± 0.000	1.079 ± 0.000
56	1.079 ± 0.000	1.079 ± 0.000	1.079 ± 0.000
59	1.082 ± 0.000	1.082 ± 0.000	1.082 ± 0.000
62	1.081 ± 0.000[a]	1.080 ± 0.000[b]	1.081 ± 0.000[ab]
65	1.078 ± 0.000	1.078 ± 0.000	1.079 ± 0.000
68	1.080 ± 0.000	1.079 ± 0.000	1.080 ± 0.000
71	1.078 ± 0.000	1.079 ± 0.000	1.080 ± 0.000
Overall	1.080 ± 0.000	1.080 ± 0.000	1.080 ± 0.000

[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage with the determination made on 2 eggs per cage collected at the end of each 3-week experimental period.

[a-b] Values within in a row with different superscripts differ, ($P < 0.05$).

Table 4.10. Haugh units of eggs produced by heat stressed hens fed different dietary selenium sources with eggs stored for 2 weeks at room temperature[1].

Hen age	Dietary treatment		
	Sodium selenite	Sel-Plex	Selisseo
wk			
44	68.21 ± 0.51	68.53 ± 0.46	68.04 ± 0.46
47	74.91 ± 0.43	74.89 ± 0.46	75.33 ± 0.40
50	73.01 ± 0.46	72.97 ± 0.45	72.42 ± 0.42
53	70.41 ± 0.43	70.81 ± 0.43	71.50 ± 0.39
56	71.19 ± 0.51	70.22 ± 0.41	69.93 ± 0.42
59	70.98 ± 0.38	71.49 ± 0.43	71.61 ± 0.39
62	71.09 ± 0.49	70.77 ± 0.43	70.72 ± 0.37
65	76.30 ± 0.49	77.21 ± 0.44	75.87 ± 0.39
68	70.98 ± 0.52	70.03 ± 0.52	70.93 ± 0.50
71	71.83 ± 0.45	71.44 ± 0.43	71.73 ± 0.47
Overall	71.94 ± 0.29	71.82 ± 0.26	71.80 ± 0.23

[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage with the determination made on 2 eggs per cage collected at the end of each 3-week experimental period.

[a-b] Values within in a row with different superscripts differ, ($P < 0.05$).

Table 4.11. Egg component weights of eggs produced by heat stressed hens fed different dietary selenium sources with eggs stored for 2 weeks at room temperature and calculations based on egg weight after 2 weeks of room temperature storage[1].

Age	Treatment	Albumen weight		Yolk weight		Shell weight	
wk		g	%	g	%	g	%
44	Sodium selenite	32.46 ± 0.22	55.70 ± 0.20	17.80 ± 0.14	30.56 ± 0.18	7.98 ± 0.07	13.73 ± 0.12
	Sel-Plex	32.80 ± 0.26	55.79 ± 0.18	17.83 ± 0.11	30.40 ± 0.15	8.10 ± 0.07	13.81 ± 0.11
	Selisseo	32.23 ± 0.28	55.38 ± 0.21	17.83 ± 0.11	30.72 ± 0.17	8.07 ± 0.06	13.91 ± 0.10
47	Sodium selenite	33.91 ± 0.27	56.07 ± 0.19	18.13 ± 0.12	30.04 ± 0.15	8.38 ± 0.07	13.89 ± 0.11
	Sel-Plex	34.07 ± 0.25	56.19 ± 0.18	18.18 ± 0.14	30.02 ± 0.17	8.34 ± 0.05	13.79 ± 0.08
	Selisseo	33.23 ± 0.26	55.81 ± 0.16	18.07 ± 0.13	30.40 ± 0.15	8.20 ± 0.08	13.79 ± 0.11
50	Sodium selenite	32.86 ± 0.26	55.42 ± 0.19	18.18 ± 0.13	30.71 ± 0.18	8.21 ± 0.07	13.87 ± 0.11
	Sel-Plex	33.01 ± 0.23	55.33 ± 0.17	18.52 ± 0.14	31.05 ± 0.16	8.11 ± 0.06	13.61 ± 0.09
	Selisseo	32.75 ± 0.24	55.22 ± 0.19	18.44 ± 0.12	31.13 ± 0.16	8.08 ± 0.07	13.65 ± 0.11
53	Sodium selenite	33.31 ± 0.26	55.16 ± 0.20	18.85 ± 0.14	31.25 ± 0.17	8.20 ± 0.07	13.59 ± 0.11
	Sel-Plex	33.27 ± 0.27	55.20 ± 0.22	18.71 ± 0.13	31.11 ± 0.17	8.24 ± 0.07	13.69 ± 0.09
	Selisseo	32.91 ± 0.28	55.19 ± 0.20	18.54 ± 0.13	31.17 ± 0.17	8.12 ± 0.08	13.64 ± 0.11
56	Sodium selenite	33.44 ± 0.27	55.02 ± 0.18	18.88 ± 0.14	31.11 ± 0.15	8.40 ± 0.06	13.86 ± 0.10
	Sel-Plex	33.69 ± 0.30	55.01 ± 0.20	19.06 ± 0.14	31.18 ± 0.16	8.43 ± 0.07	13.80 ± 0.10
	Selisseo	32.80 ± 0.26	54.56 ± 0.20	18.95 ± 0.14	31.56 ± 0.17	8.32 ± 0.06	13.88 ± 0.10
59	Sodium selenite	33.91 ± 0.26	55.23 ± 0.19	19.15 ± 0.14	31.24 ± 0.17	8.29 ± 0.07	13.53 ± 0.09
	Sel-Plex	34.06 ± 0.27	55.52 ± 0.20	19.03 ± 0.15	31.04 ± 0.18	8.24 ± 0.08	13.45 ± 0.10

	Selisseo	33.66 ± 0.31	55.43 ± 0.22	18.74 ± 0.14	30.93 ± 0.18	8.26 ± 0.07	13.64 ± 0.10
62	Sodium selenite	34.86 ± 0.30	55.89 ± 0.18	18.98 ± 0.15	30.46 ± 0.15	8.49 ± 0.07	13.64 ± 0.10
	Sel-Plex	34.53 ± 0.27	55.42 ± 0.21	19.11 ± 0.14	30.70 ± 0.16	8.63 ± 0.08	13.88 ± 0.12
	Selisseo	33.91 ± 0.32	55.92 ± 0.19	19.01 ± 0.15	30.51 ± 0.17	8.46 ± 0.08	13.57 ± 0.10
65	Sodium selenite	34.69 ± 0.30	55.67 ± 0.20	19.15 ± 0.14	30.80 ± 0.17	8.42 ± 0.09	13.53 ± 0.12
	Sel-Plex	35.00 ± 0.27	55.66 ± 0.20	19.34 ± 0.16	30.79 ± 0.19	8.51 ± 0.09	13.55 ± 0.12
	Selisseo	34.64 ± 0.30	55.44 ± 0.19	19.37 ± 0.16	31.05 ± 0.17	8.42 ± 0.07	13.51 ± 0.11
68	Sodium selenite	33.46 ± 0.28^{ab}	54.74 ± 0.20	19.14 ± 0.14	31.36 ± 0.16	8.48 ± 0.08	13.90 ± 0.12
	Sel-Plex	34.31 ± 0.28^{a}	54.97 ± 0.20	19.55 ± 0.17	31.34 ± 0.18	8.53 ± 0.08	13.69 ± 0.11
	Selisseo	33.31 ± 0.29^{b}	54.50 ± 0.19	19.37 ± 0.15	31.75 ± 0.16	8.38 ± 0.08	13.75 ± 0.11
71	Sodium selenite	33.87 ± 0.28	54.38 ± 0.21	19.91 ± 0.16	32.00 ± 0.19	8.48 ± 0.09	13.63 ± 0.12
	Sel-Plex	34.23 ± 0.29	54.49 ± 0.22	20.01 ± 0.16	31.90 ± 0.20	8.54 ± 0.08	13.61 ± 0.11
	Selisseo	34.34 ± 0.34	54.54 ± 0.21	20.06 ± 0.15	31.97 ± 0.19	8.48 ± 0.09	13.50 ± 0.11
Overall	Sodium selenite	33.66 ± 0.16	55.32 ± 0.12	18.81 ± 0.08	30.95 ± 0.10	8.34 ± 0.04	13.73 ± 0.05
	Sel-Plex	33.84 ± 0.16	55.34 ± 0.12	18.92 ± 0.08	30.97 ± 0.10	8.36 ± 0.04	13.69 ± 0.05
	Selisseo	33.44 ± 0.17	55.19 ± 0.11	18.82 ± 0.15	31.11 ± 0.10	8.28 ± 0.04	13.69 ± 0.05

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[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage with the determination made on one egg per cage collected at the end of each 3-week experimental period. $^{a\text{-}b}$Values within in a row with different superscripts differ, ($P < 0.05$)

Table 4.12. Yolk color of eggs produced by heat stressed hens fed different dietary selenium sources with eggs stored for 2 weeks at room temperature[1].

Age	Treatment	Yolk color		
wk		L[2]	a*[3]	b*[4]
44	Sodium selenite	61.48 ± 0.32	5.66 ± 0.18	33.24 ± 0.20
	Sel-Plex	61.21 ± 0.33	5.60 ± 0.14	33.15 ± 0.20
	Selisseo	61.09 ± 0.31	5.67 ± 0.14	32.98 ± 0.20
47	Sodium selenite	63.70 ± 0.34	5.72 ± 0.13	33.65 ± 0.22
	Sel-Plex	63.97 ± 0.35	5.66 ± 0.13	33.84 ± 0.23
	Selisseo	63.64 ± 0.37	5.54 ± 0.13	33.78 ± 0.24
50	Sodium selenite	62.48 ± 0.32	-4.98 ± 0.11	33.41 ± 0.24
	Sel-Plex	63.02 ± 0.35	-5.30 ± 0.13	32.75 ± 0.28
	Selisseo	62.63 ± 0.36	-5.23 ± 0.10	33.31 ± 0.24
53	Sodium selenite	68.73 ± 0.32	-0.86 ± 0.12	56.28 ± 0.42
	Sel-Plex	68.84 ± 0.35	-0.70 ± 0.14	56.00 ± 0.44
	Selisseo	69.25 ± 0.34	-0.58 ± 0.15	56.59 ± 0.45
56	Sodium selenite	69.79 ± 0.32	-0.22 ± 0.12	56.96 ± 0.35
	Sel-Plex	69.43 ± 0.37	-0.18 ± 0.13	56.98 ± 0.39
	Selisseo	69.75 ± 0.35	-0.17 ± 0.11	57.36 ± 0.36
59	Sodium selenite	68.71 ± 0.34	-0.09 ± 0.15	54.09 ± 0.42
	Sel-Plex	68.88 ± 0.34	0.13 ± 0.14	54.03 ± 0.49
	Selisseo	68.79 ± 0.39	0.12 ± 0.14	54.18 ± 0.40
62	Sodium selenite	68.60 ± 0.32	0.32 ± 0.19	51.60 ± 0.50
	Sel-Plex	69.25 ± 0.27	0.33 ± 0.15	51.85 ± 0.43
	Selisseo	69.22 ± 0.28	0.68 ± 0.14	52.33 ± 0.45
65	Sodium selenite	69.12 ± 0.26	0.47 ± 0.18	51.47 ± 0.48
	Sel-Plex	69.10 ± 0.29	0.41 ± 0.18	51.92 ± 0.48
	Selisseo	69.70 ± 0.27	0.31 ± 0.16	50.84 ± 0.49

68	Sodium selenite	68.80 ± 0.41	-0.53 ± 0.13	52.62 ± 0.40
	Sel-Plex	68.73 ± 0.35	-0.16 ± 0.14	53.03 ± 0.53
	Selisseo	69.21 ± 0.33	-0.42 ± 0.15	52.86 ± 0.45
71	Sodium selenite	67.98 ± 0.33	0.01 ± 0.12	53.92 ± 0.44
	Sel-Plex	67.98 ± 0.36	-0.14 ± 0.12	54.29 ± 0.46
	Selisseo	67.86 ± 0.32	-0.04 ± 0.12	54.39 ± 0.40
Overall	Sodium selenite	66.82 ± 0.13	0.66 ± 0.05	47.45 ± 0.17
	Sel-Plex	66.92 ± 0.14	0.66 ± 0.06	47.41 ± 0.18
	Selisseo	67.03 ± 0.13	0.66 ± 0.05	47.60 ± 0.15

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[1] Values are means ± SEM, n=120 cages per treatment with 2 hens per cage with the determination made on one egg per cage collected at the end of each 3-week experimental period.

[2] L* is a measure of the difference in lightness and darkness with lightness registering as a more positive number.

[3] a* is a measure of the difference in red and green, with redness registering as a more positive number.

[4] b* is a measure of the difference in yellow and blue, with yellowness registering as a more positive number.

Table 4.13. Selenium content of eggs on a dry matter basis from heat stressed hens fed different dietary selenium sources[1]

Age	Treatment	Egg Se content
Wk		ug/g
41 (pre-experiment	Sodium selenite	1.204 ± 0.010
	Sel-Plex	1.201 ± 0.007
	Selisseo	1.217 ± 0.013
47	Sodium selenite	1.215 ± 0.011^{b}
	Sel-Plex	1.271 ± 0.017^{b}
	Selisseo	1.981 ± 0.020^{a}
53	Sodium selenite	1.155 ± 0.018^{b}
	Sel-Plex	1.223 ± 0.018^{b}
	Selisseo	1.729 ± 0.029^{a}
59	Sodium selenite	1.213 ± 0.023^{b}
	Sel-Plex	1.242 ± 0.024^{b}
	Selisseo	1.810 ± 0.013^{a}
65	Sodium selenite	0.978 ± 0.012^{b}
	Sel-Plex	1.018 ± 0.011^{b}
	Selisseo	1.585 ± 0.030^{a}
71	Sodium selenite	0.995 ± 0.025^{b}
	Sel-Plex	1.060 ± 0.023^{b}
	Selisseo	1.487 ± 0.038^{a}

[1] Values are means ± SEM, n=8 blocks per treatment with each block consisting of 10 cages with each cage containing 2 hens. Three eggs were selected from each block with the yolk and albumen from the 3 eggs combined, homogenized and freeze dried. $^{a\text{-}b}$Values within in a row for a given interval with different superscripts differ, ($P < 0.000$).

Table 4.14. Feed consumed relative to egg production of heat stressed hens fed different dietary selenium sources[1].

Age	Treatment	Individual 3 week interval			Cumulative experimental period		
(wk)		Feed/egg (kg/kg)	Feed/dozen eggs	Feed/egg (kg feed/total # eggs produced	Feed/egg (kg/kg)	Feed/dozen eggs	Feed/egg (kg feed/total # eggs produced
42-44	Selenite	2.051 ± 0.019^{a}	1.479 ± 0.012	0.123 ± 0.001	2.051 ± 0.019^{a}	1.479 ± 0.012	0.123 ± 0.001
	Sel-Plex	1.993 ± 0.011^{b}	1.448 ± 0.007	0.121 ± 0.001	1.993 ± 0.011^{b}	1.448 ± 0.007	0.121 ± 0.001
	Selisseo	2.020 ± 0.014^{ab}	1.453 ± 0.012	0.121 ± 0.001	2.020 ± 0.014^{ab}	1.453 ± 0.012	0.121 ± 0.001
45-47	Selenite	2.240 ± 0.025	1.637 ± 0.018	0.136 ± 0.002	2.144 ± 0.020	1.557 ± 0.014	0.130 ± 0.001
	Sel-Plex	2.209 ± 0.017	1.620 ± 0.016	0.135 ± 0.001	2.099 ± 0.009	1.532 ± 0.009	0.128 ± 0.001
	Selisseo	2.208 ± 0.013	1.611 ± 0.010	0.134 ± 0.001	2.118 ± 0.011	1.531 ± 0.010	0.128 ± 0.001
48-50	Selenite	2.161 ± 0.026	1.594 ± 0.020	0.133 ± 0.002	2.149 ± 0.021	1.569 ± 0.015	0.131 ± 0.001
	Sel-Plex	2.154 ± 0.017	1.593 ± 0.015	0.133 ± 0.001	2.117 ± 0.011	1.552 ± 0.011	0.129 ± 0.001
	Selisseo	2.120 ± 0.015	1.558 ± 0.012	0.130 ± 0.001	2.118 ± 0.010	1.540 ± 0.010	0.128 ± 0.001
51-53	Selenite	2.178 ± 0.029^{a}	1.622 ± 0.022^{a}	0.135 ± 0.002^{a}	2.156 ± 0.022	1.581 ± 0.016	0.132 ± 0.001
	Sel-Plex	2.150 ± 0.014^{ab}	1.607 ± 0.009^{ab}	0.134 ± 0.001^{ab}	2.125 ± 0.010	1.565 ± 0.010	0.130 ± 0.001
	Selisseo	2.102 ± 0.013^{b}	1.558 ± 0.012^{b}	0.130 ± 0.001^{b}	2.115 ± 0.009	1.544 ± 0.010	0.129 ± 0.001
54-56	Selenite	2.185 ± 0.024	1.638 ± 0.017^{a}	0.136 ± 0.001^{a}	2.162 ± 0.021	1.592 ± 0.015	0.133 ± 0.001
	Sel-Plex	2.159 ± 0.019	1.629 ± 0.016^{ab}	0.136 ± 0.001^{ab}	2.132 ± 0.011	1.577 ± 0.010	0.131 ± 0.001
	Selisseo	2.117 ± 0.023	1.581 ± 0.016^{b}	0.132 ± 0.001^{b}	2.115 ± 0.011	1.551 ± 0.010	0.129 ± 0.001
57-59	Selenite	2.206 ± 0.029^{a}	1.663 ± 0.021^{a}	0.139 ± 0.002^{a}	2.168 ± 0.020^{a}	1.603 ± 0.015^{a}	0.134 ± 0.001^{a}

	Sel-Plex	2.132 ± 0.019^{ab}	1.617 ± 0.013^{ab}	0.135 ± 0.001^{ab}	2.132 ± 0.011^{ab}	1.583 ± 0.010^{ab}	0.132 ± 0.001^{ab}
	Selisseo	2.121 ± 0.020^{b}	1.594 ± 0.017^{b}	0.133 ± 0.001^{b}	2.116 ± 0.011^{b}	1.558 ± 0.010^{b}	0.130 ± 0.001^{b}
60-62	Selenite	2.244 ± 0.024^{a}	1.711 ± 0.015^{a}	0.143 ± 0.001^{a}	2.179 ± 0.020^{a}	1.617 ± 0.014^{a}	0.135 ± 0.001^{a}
	Sel-Plex	2.201 ± 0.023^{ab}	1.681 ± 0.016^{ab}	0.140 ± 0.001^{ab}	2.141 ± 0.012^{ab}	1.596 ± 0.010^{ab}	0.133 ± 0.001^{ab}
	Selisseo	2.144 ± 0.022^{b}	1.631 ± 0.018^{b}	0.136 ± 0.002^{b}	2.120 ± 0.011^{b}	1.567 ± 0.010^{b}	0.131 ± 0.001^{b}
63-65	Selenite	2.165 ± 0.025^{ab}	1.658 ± 0.018^{ab}	0.138 ± 0.002^{ab}	2.177± 0.020^{a}	1.622 ± 0.014^{a}	0.135 ± 0.001^{a}
	Sel-Plex	2.193 ± 0.023^{a}	1.690 ± 0.016^{a}	0.141 ± 0.001^{a}	2.147 ± 0.012^{ab}	1.607 ± 0.010^{ab}	0.134 ± 0.001^{ab}
	Selisseo	2.093 ± 0.027^{b}	1.602 ± 0.023^{b}	0.134 ± 0.002^{b}	2.116 ± 0.011^{b}	1.571 ± 0.011^{b}	0.131 ± 0.001^{b}
66-68	Selenite	2.288 ± 0.025	1.750 ± 0.020	0.146 ± 0.002	2.189 ± 0.019^{a}	1.635 ± 0.014^{a}	0.136 ± 0.001^{a}
	Sel-Plex	2.279 ± 0.033	1.754 ± 0.026	0.146 ± 0.002	2.161 ± 0.013^{ab}	1.622 ± 0.010^{ab}	0.135 ± 0.001^{ab}
	Selisseo	2.240 ± 0.022	1.706 ± 0.017	0.142 ± 0.001	2.129 ± 0.011^{b}	1.585 ± 0.011^{b}	0.132 ± 0.001^{b}
69-71	Selenite	2.429 ± 0.036^{a}	1.865 ± 0.029^{a}	0.155 ± 0.002^{a}	2.211 ± 0.020^{a}	1.656 ± 0.015^{a}	0.138 ± 0.001^{a}
	Sel-Plex	2.370 ± 0.035^{ab}	1.832 ± 0.025^{ab}	0.153 ± 0.002^{ab}	2.180 ± 0.014^{ab}	1.641 ± 0.010^{ab}	0.137 ± 0.001^{ab}
	Selisseo	2.312 ± 0.024^{b}	1.774 ± 0.019^{b}	0.148 ± 0.002^{b}	2.146 ± 0.012^{b}	1.603 ± 0.011^{b}	0.134 ± 0.001^{b}

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[1] Values are means ± SEM, n=12 blocks per treatment with each block consisting of 10 cages with each cage containing 2 hens. $^{a-b}$Values within in a row for a given interval with different superscripts differ, ($P < 0.05$).

Table 4.15. Liver score and liver color of 72 week old hens that had been heat stressed from 42 to 71 weeks of age and fed diets containing different selenium sources[1].

Treatment	Liver score[2]	L*[3]	a*[4]	b*[5]
Sodium selenite	5.95 ± 0.24	46.17± 1.10	15.74 ± 0.28	20.27 ± 0.96
Sel-Plex	6.04 ± 0.26	44.92 ± 0.66	16.20 ± 0.28	19.65 ± 0.76
Selisseo	6.18 ± 0.22	44.95 ± 0.59	16.05 ± 0.29	19.53 ± 0.73

[1] The values are means ± SEM, n = 48 replicate hens. Four hens were selected from each of the 12 blocks per treatment. The hens selected were the closest to the average egg production for each block in the preceding 2 weeks prior to sampling at the end of the experiment. pens with 8 birds per pen selected for processing

[2] The liver score was based on a visual and tissue assessment with scores ranging from 1 to 10. A score of 1 was given to a liver that had a rich brown red color for the entire surface, had no visual lesions or hemorrhages and whose tissue remained intact as handled. A score of 10 was given to a liver that had a light tan color for the entire surface indicating complete infiltration by lipid, had widespread lesions and/or hemorrhages and whose tissue completely disintegrated when handled.

[3] L* is a measure of the difference in lightness and darkness with lightness registering as a more positive number.

[4] a* is a measure of the difference in red and green, with redness registering as a more positive number.

[5] b* is a measure of the difference in yellow and blue, with yellowness registering as a more positive number.

Table 4.16. Malondialdehyde content of liver and serum collected from 72 week old laying hens that had been heat stressed from 42 to 71 weeks of age and fed diets containing different selenium sources[1].

Treatment	Liver	Serum
	uM	
Sodium selenite	15.47 ± 2.49	2.73 ± 0.19
Sel-Plex	15.42 ± 2.53	2.80 ± 0.17
Selisseo	13.24 ± 1.68	2.97 ± 0.15

[1] The values are means ± SEM, n = 24 replicate cages with each cage containing 2 hens. Two cages were selected from each of the 12 blocks per treatment with the 2 selected cages being those closest to the mean egg production rate for the bock.

CONCLUSIONS

As the availability and utilization of purified nutrients in poultry diets has increased, the overall composition of these diets has concomitantly become more defined. However, with the reduction of natural ingredients, these diets have potentially lost natural sources of trace elements that serve imperative functions in metabolism and assist with such things as immune function and antioxidant status of the bird. In turn, the essentiality of certain nutrients may come to light as either their absence or suboptimal inclusion levels in the diet may impact performance. In the current research, supplementation of layer and broiler breeder hen diets with Azomite which contains a wide variety of rare earth elements significantly improved marketable and settable egg production, respectively. The current research also indicates that dietary Azomite supplementation improves calcium absorption in broilers and tibia ash content in laying hens. The mechanisms by which Azomite supplementation improved egg production so significantly in the current research is unclear, but its potential to alter the stress response warrants further investigation. Although Azomite supplementation did not reduce the rise in serum AGP (one potential biomarker for stress) concentration while molting the laying hens, the Azomite-supplemented hens returned to lay quicker and the greatest gain in egg production was observed post-molt relative to control hens fed a diet not supplemented with Azomite. Broiler breeder hens are exposed to the stresses of feed restriction, reproduction, and the social stress of a floor pen breeding flock. Thus, the 9 egg per hen increase in settable eggs in the Azomite supplemented hens could again be due to improved stress management in these hens.

Moving towards greater defined diets is not only significant with respect to the loss of certain components such as rare earth elements, but it also highlights the relevance of the sources of trace elements that are used. For instance, in major feed ingredients such as grains and oilseed, trace elements such as selenium are present in organic forms, whereas inorganic forms of selenium have long dominated the form provided via commercial supplements. However, the results of the current research agree with previous research indicating that the efficacy of inorganic and organic mineral sources vary. This is not only significant with respect to optimizing bird performance under ideal conditions, but also to mitigating distress and maximizing bird performance under conditions that commonly plague the poultry industry, such as high stocking density and heat stress. In the current research, based on significantly improved egg production in heat stressed hens fed a diet supplemented with the organic selenium source Selisseo® versus an inorganic source (sodium selenite) and another less compositionally defined source of organic selenium, the Selisseo®-fed hens were better able to mitigate the effects of heat stress. Heat stress induces oxidative stress, and future investigations will benefit by examining multiple measures of oxidative stress, such as selenoprotein activities, as plasma and liver malondialdehyde production was not reduced by Selisseo® supplementation in the current research.

In conclusion, further research is necessary to elucidate the essentiality of rare earth elements and their respective dietary inclusion levels, as well as further defining the dietary requirements of known dietary essential trace elements particularly under conditions of stress. This research, along with previous research, have provided evidence that the physiological effects of these trace elements may be magnified under stressful conditions, and that organic sources of trace minerals may be advantageous over inorganic sources for optimal animal performance.