# IMPACT OF SEASONAL CHANGES IN PASTURE-RAISING SYSTEMS ON EGG NUTRITION

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## ABSTRACT

Pasture-raised egg production is increasingly recognized for its alignment with regenerative agriculture, emphasizing biodiversity, soil health, and ethical animal husbandry. These systems allow laying hens to forage on diverse plant species and insects, which can significantly improve the nutrient composition of eggs compared to conventional grain-based systems. However, the nutrient profile of pasture-raised eggs may fluctuate due to seasonal variation in forage availability, quality, and environmental conditions.

This study aimed to evaluate how seasonal changes, specifically late spring through early winter, affect the nutrient density of eggs produced in a pasture-based system in Southern Ohio. Monthly collections of forage, soil, and eggs were conducted from May to December 2022. Nutrient composition of egg yolks was assessed using gas chromatography–mass spectrometry (GC-MS), liquid chromatography, spectrophotometry, and inductively coupled plasma optical emission spectroscopy (ICP-OES). Nutrients analyzed included carotenoids, vitamins A and E, total phenolics, and fatty acids.

Findings revealed that forage quality, as measured by total digestible nutrients (TDN), peaked in October, aligning with increased deposition of omega-3 fatty acids and fat-soluble antioxidants in egg yolks during fall months. Principal component and random forest analyses identified September to November as the separated time period yielding the most nutrient-dense eggs. This research supports the role of pasture-raised systems in delivering nutrient-rich animal products and highlights the need for seasonal considerations in both consumer education and on-farm management strategies.

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## 1.1 Introduction

Eggs are one of the most widely consumed animal-derived foods globally and are a staple in many diets due to their affordability, versatility, and dense nutrient profile (Farrell, 2013; Headey & Alderman, 2019). They provide high-quality protein, essential fatty acids, fat-soluble vitamins, and carotenoids, making them an important dietary component for human health (Nimalaratne & Wu, 2015; Usturoi et al., 2025). Because eggs are a primary source of these essential nutrients, they have the potential to play a significant role in dietary modifications aimed at improving overall health outcomes (Cartoni Mancinelli et al., 2022; Headey & Alderman, 2019).

The nutritional composition of eggs is influenced by various factors, including the hen's diet, production system, and environmental conditions (Lantzouraki, 2020). Research has shown that pasture-raised eggs, in particular, have enhanced fatty acid and antioxidant profiles compared to conventional eggs (Ben-Noun, 2019; Sergin et al., 2021). This improvement is especially relevant for correcting dietary imbalances in the modern American diet, which is often high in saturated fat and has an unfavorable n-6:n-3 ratio (Simopoulos, 2008). By focusing on improving the fatty acid composition of eggs through production methods, eggs could serve as a key food in dietary strategies aimed at reducing chronic inflammation and improving cardiovascular health (Mwai, 2021; Wang et al., 2024).

Eggs from different production systems vary significantly in nutrient composition (Ben-Noun, 2019). Conventional egg production relies on grain-based diets, typically composed of corn and soy, which are rich in omega-6 polyunsaturated fatty acids (n-6 PUFAs) but lack beneficial omega-3 (n-3) PUFAs and antioxidants (Clancy, 2006). In contrast, pasture-raised egg systems provide

hens with access to forage, insects, and diverse plant species, producing eggs with significantly improved nutrient profiles (Sergin et al., 2021). Compared to conventional eggs, pasture-raised eggs have been shown to contain twice the vitamin E content and up to 2.5 times more n-3 PUFAs, resulting in a more favorable n-6:n-3 ratio (Sergin et al., 2021). This is particularly relevant for cardiovascular health, as an excessive n-6:n-3 ratio has been linked to chronic inflammation and metabolic diseases (Simopoulos, 2008; Wang et al., 2024).

## 1.2 How Pasture-Raised Eggs Align with Human Health

### *1.2.1 Health Rationale*

The American diet is characterized by a high intake of saturated fat and an imbalanced n-6:n-3 ratio, which has been linked to increased inflammation and chronic disease risk (Mariamenatu & Abdu, 2021; Simopoulos, 2008). Because eggs are widely consumed and serve as a staple protein source, they represent an ideal food for improving dietary fatty acid profiles (Ben-Noun, 2019). By enhancing the nutrient composition of eggs, specifically increasing omega-3 content and optimizing the n-6:n-3 ratio, producers can contribute to improved public health outcomes (Patel et al., 2022; Usturoi et al., 2025). By modifying feed and forage access, producers can create a more favorable lipid profile in eggs, offering a simple and effective dietary intervention to address nutritional imbalances in the modern diet (Patel et al., 2022).

The bioavailability of these nutrients is crucial, as their dietary sources vary in absorption efficiency (Zaheer, 2017). Eggs, due to their high natural fat content, enhance the absorption of fat-soluble nutrients such as carotenoids (Schweiggert & Carle, 2017). This is because eggs contain a naturally high fat content, which facilitates the absorption of fat-soluble nutrients like carotenoids, whereas forage species, despite being rich in these compounds, lack sufficient fat content to optimize their bioavailability (Moreno et al., 2016; Ren et al., 2010). Therefore,

improving the nutrient content of eggs through hen dietary interventions can provide a more accessible and bioavailable source of these essential nutrients (Goldberg et al., 2016; Vlaicu & Untea, 2024).

### *1.2.2 Fatty Acids, Phytochemicals, and Human Health*

Fatty acids and phytochemicals found in eggs have distinct health benefits that support cardiovascular, neurological, and metabolic functions (Saidaiah et al., 2024). These compounds originate from different dietary sources and play key roles in biological processes, making them crucial for maintaining health.

Fatty acids are essential components of cell membranes and play a fundamental role in energy metabolism and inflammatory responses (Simopoulos, 2008). Omega-3 fatty acids, including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), are known for their cardioprotective effects, reducing triglyceride levels, lowering blood pressure, and supporting cognitive function (D'Angelo et al., 2020). ALA, primarily found in plant sources such as flaxseed and leafy greens, is converted in the body to EPA and DHA, although the conversion is limited (Management et al., 2023). EPA and DHA, which are mainly derived from marine sources, are crucial for brain development and neuroprotection (Simopoulos, 2008). In contrast, omega-6 fatty acids, such as linoleic acid (LA) and arachidonic acid (AA), are commonly found in vegetable oils and grains. While they are essential for inflammatory and immune responses, an excessively high n-6:n-3 ratio has been associated with chronic inflammation, emphasizing the importance of dietary balance (Harris et al., 2009; Oluwole et al., 2019).

Carotenoids are pigmented compounds found in plants that function as antioxidants and serve as precursors to vitamin A (Zielińska-Dawidziak et al., 2024). Lutein and zeaxanthin, concentrated in leafy greens and egg yolks, play a critical role in eye health by reducing the risk of age-related

macular degeneration and filtering harmful blue light (Lantzouraki, 2020). β-Carotene, present in carrots, sweet potatoes, and forage plants, is converted into retinol (vitamin A), which is essential for immune function, vision, and cellular differentiation (Zielińska-Dawidziak et al., 2024). Unlike direct vitamin A sources, β-carotene requires enzymatic conversion, making dietary intake an important determinant of its bioavailability (D'Archivio et al., 2010).

Phenolic compounds, including flavonoids and polyphenols, are bioactive compounds found in plant-based foods such as fruits, vegetables, and forage crops. Unlike carotenoids, phenolics are non-nutritive but exert significant health benefits through their antioxidant and anti-inflammatory properties (D'Archivio et al., 2010). These compounds protect cells from oxidative stress by scavenging reactive oxygen species (ROS), reducing the risk of chronic diseases such as cardiovascular disease and cancer (Sergin et al., 2022).

A key factor influencing the bioactivity of these compounds is their dietary source and matrix. Pasture-raised eggs deliver a superior antioxidant and fatty acid profile due to hens' access to nutrient-dense forages rich in omega-3s, carotenoids, and polyphenols (Krusinski, Maciel, et al., 2022). As a result, pasture-raised eggs serve as a valuable dietary source of bioavailable fatty acids and phytochemicals, contributing to improved cardiovascular, cognitive, and metabolic health (Sergin et al., 2022).

### 1.3 Overview of Fatty Acids and Antioxidants in Egg Yolks

Egg yolks contain a rich profile of fatty acids, antioxidants, and essential vitamins, all of which contribute to their nutritional value. The fatty acid composition of egg yolks is predominantly made up of monounsaturated fatty acids (MUFAs), which account for approximately 45% of total lipids, with oleic acid (C18:1) being the most abundant MUFA (Agriculture, 2019). Saturated fatty acids (SFAs) represent a slightly lower proportion, while polyunsaturated fatty acids (PUFAs),

including omega-6 and omega-3 fatty acids, make up the smallest fraction of total lipid content and is subject to variation (Agriculture, 2019).

Among the omega-3 fatty acids present in egg yolks are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The proportions of these fatty acids vary based on the hen's diet, with ALA typically being the most prevalent in pasture-raised eggs. On average, a conventional egg contains about 30–50 mg of total omega-3s, while pasture-raised or enriched eggs may contain upwards of 150–200 mg per egg, depending on feed composition and access to forages (Sergin et al., 2021; Sergin et al., 2022). DHA is particularly important for human health, and its concentration in enriched or pasture-raised eggs can range from 50–150 mg per yolk, compared to less than 20 mg in conventional systems (Sergin et al., 2021; Sergin et al., 2022).

Egg yolks are a valuable dietary source of several fat-soluble nutrients, particularly carotenoids and vitamins A and E. According to USDA reference values, conventional egg yolks contain approximately 371 μg of retinol (preformed vitamin A) and 2.58 mg of vitamin E (α-tocopherol) per yolk. In addition, they provide a modest amount of provitamin A carotenoids, with a combined concentration of approximately 126 μg of total carotene (including β-carotene and α-carotene), which can be enzymatically converted into retinol in the human body (Agriculture, 2019). Small amounts of phenolic compounds may also be present, though these are typically limited due to their hydrophilic structure, which reduces deposition into the lipid-dense yolk matrix (Agriculture, 2019; Sergin et al., 2021).

Cholesterol levels in eggs remain relatively stable, typically ranging from 180–230 mg per egg, regardless of dietary modifications (Attia et al., 2022). While feed modifications can influence

yolk fatty acid and antioxidant levels, cholesterol is regulated metabolically and not directly by diet (Vlaicu et al., 2021).

Eggs serve as a nutrient-dense source of essential fatty acids, vitamins, and antioxidants, making them a key component of a balanced diet. However, their nutritional composition can be significantly influenced by modifying hen diets, particularly when they have access to forages rich in bioactive compounds.

### 1.4 Nutrient Differences Between Egg Production Systems

While eggs naturally contain essential fatty acids, vitamins, and antioxidants, significant differences in nutrient composition have been observed between various egg production systems. Studies comparing conventionally produced, cage-free, organic, and pasture-raised eggs offer superior levels of omega-3s, carotenoids, vitamin A, and vitamin E (Oke & Onagbesan, 2013; Sergin et al., 2022).

These differences are largely attributed to dietary access. Conventionally raised hens are typically fed corn- and soy-based rations, which are high in omega-6 fatty acids and low in omega-3s—leading to an unfavorable n-6:n-3 ratio in the yolk. These eggs may contain as little as 30 mg of total omega-3s and a n-6:n-3 ratio exceeding 15:1 (Sergin et al., 2022). Cage-free and organic systems may incorporate minor dietary variations, but they do not consistently provide the forage access necessary to significantly enhance bioactive nutrient content (Bist et al., 2024; Nopparatmaitree et al., 2022).

In contrast, pasture-raised systems allow hens to forage freely, granting access to a diverse range of plants, insects, and soil-based nutrients (Bist et al., 2024). Carotenoid-rich forage species, such as clover, alfalfa, and grasses, contribute to deeper yolk pigmentation, while access to fresh vegetation increases vitamin E concentrations (Krusinski, Maciel, et al., 2022). Notably, pasture-

raised is the only system that mandates outdoor access for hens, unlike cage-free or free-range models where such access is not always enforced (Humane, 2014).

As a result, pasture-raised eggs often contain 2 to 3 times more total omega-3 fatty acids than conventional eggs (H. Karsten et al., 2010). In some cases, pasture-raised eggs contain over 100 mg of DHA per yolk, contributing to a significantly lower n-6:n-3 ratio, often approaching 2:1 to 4:1 (H. Karsten et al., 2010; Sergin et al., 2021; Sergin et al., 2022). Pasture-raised eggs have also been shown to contain over 60 μg/g of total carotenoids, compared to as low as 15 μg/g in conventional eggs (Sergin et al., 2021; Sergin et al., 2022). Vitamin E content was also markedly higher, with pasture-raised eggs containing up to 2 to 3 times the amount of alpha-tocopherol compared to conventional production systems (Sergin et al., 2021; Sergin et al., 2022).

This distinction highlights the nutritional significance of studying pasture-raised eggs, particularly due to their hens' enhanced access to natural dietary inputs (Sergin et al., 2022). As nutrient composition is strongly influenced by production system, pasture-based models offer a unique opportunity to improve egg quality through exposure to forage-rich environments (Cristea et al., 2024). By increasing access to bioactive compounds such as carotenoids, vitamin E, and omega-3 fatty acids, pasture-raising practices contribute to eggs with superior nutritional value and potential health benefits for consumers (Oke & Onagbesan, 2013; Zielińska-Dawidziak et al., 2024).

## 1.5 Influences of Yolk Nutrition

### *1.5.1 Diet is the largest influence of nutrient variations*

Compared to conventionally produced eggs, pasture-raised eggs have been shown to contain higher levels of omega-3 fatty acids, ALA, DHA, and EPA, as a result of the hens' access to diverse forage species (Sergin et al., 2021). These beneficial fatty acids are primarily derived from

omega-3-rich plants such as clover and alfalfa, which are abundant in pasture environments (Javed et al., 2025). Hen diet remains the most critical determinant of egg nutrient composition. Commercial poultry diets, typically composed of corn and soybean meal, provide energy and protein but are deficient in omega-3 fatty acids and antioxidant compounds (Zielińska-Dawidziak et al., 2024) .

In contrast, pasture-raised hens consume a variety of forage species that serve as natural sources of phytochemicals, enhancing the nutritional profile of their eggs. Forage plants such as clover, alfalfa, and mixed grasses are rich in bioactive compounds like carotenoids, polyphenols, and flavonoids. These act as antioxidants, protecting egg yolks from oxidative degradation and contributing to a superior nutritional profile (Lantzouraki, 2020). Carotenoids such as lutein and zeaxanthin, in particular, contribute to yolk pigmentation and serve as precursors to vitamin A, which supports vision and immune function (Dansou et al., 2023). Polyphenols, meanwhile, have been associated with anti-inflammatory activity and cellular protection against oxidative stress, potentially contributing to the enhanced health benefits of pasture-raised eggs (Sergin et al., 2022).

In addition to forage-derived nutrients, hens may be provided with specialized supplemental feeds to further optimize egg composition. Standard layer rations typically include corn, wheat, and soybean meal, which provide protein and essential amino acids (Bist et al., 2024). However, feed additives such as flaxseed, fish oil, and microalgae are often incorporated to improve omega-3 content. Flaxseed is a rich source of ALA, a short-chain omega-3 that can be endogenously converted to DHA and EPA, while fish oil and microalgae directly supply long-chain omega-3s (Javed et al., 2025; Panaite et al., 2021).

Additional strategies to enhance egg quality include incorporating antioxidant-rich ingredients like marigold petals and red pepper into hen diets (Matache et al., 2024). These

ingredients are high in carotenoids and have been shown to increase their deposition in yolks, further improving pigmentation and nutritional value (Panaite et al., 2021).

### *1.5.2 Other Influence in Egg Production*

Beyond diet, factors such as hen breed, age, and laying cycle have been proposed to influence egg nutrient composition. While some studies have suggested that breed may affect omega-3 deposition and antioxidant content due to differences in metabolism or feed utilization efficiency, current evidence remains mixed (Attia et al., 2022; Kojima et al., 2022). Breed-specific effects appear to play a more substantial role in eggshell characteristics and production rate than in yolk nutrient composition (Henry, 2019).

Hen age is another important factor. Younger hens typically produce eggs with higher concentrations of vitamins and essential fatty acids. As hens age, nutrient density in eggs tends to decline, likely due to age-related changes in nutrient absorption and allocation (Gao et al., 2021). Moreover, during the hen's laying cycle, especially in its early stages, lipid metabolism is more efficient, contributing to richer yolk lipid profiles than those produced later in the cycle (Usturoi et al., 2025).

Environmental factors such as temperature and seasonal variation significantly influence egg nutrient composition by affecting hen metabolism and nutrient allocation (Pawar et al., 2016). The optimal laying temperature for hens ranges between 16°C and 22°C. When temperatures rise above this range, heat stress often reduces feed intake, resulting in lower deposition of omega-3 fatty acids, antioxidants, and vitamin E in yolks (Saleh et al., 2021; Usturoi et al., 2025). Conversely, cold stress in winter redirects metabolic energy toward thermoregulation, compromising nutrient transfer to eggs (Evaris et al., 2019; Pawar et al., 2016). To mitigate these seasonal effects,

producers may adjust feed formulations throughout the year to help maintain consistent yolk quality.

## 1.6 Seasonal Variability in Pasture-Raised Egg Nutrition

### *1.6.1 Defining Seasonal Variations*

Seasonal variation significantly influences the nutrient composition of pasture-raised eggs due to fluctuations in forage availability, plant maturity, and environmental conditions. As in beef and dairy production, seasonal changes in pasture quality affect the fatty acid, antioxidant, and vitamin content of animal-derived foods, including eggs (Krusinski, Maciel, et al., 2022). These fluctuations are driven by plant growth stages, regrowth cycles, temperature, and precipitation, which collectively alter the nutrient density of the forages hens consume.

Forage is most nutrient-rich in its early growth stages, when leaf-to-stem ratios are high, resulting in greater concentrations of ALA, carotenoids, and vitamins (Chatzidimitriou, 2020). As plants mature, they lignify, reducing digestibility and nutrient availability. These changes, well-studied in ruminant nutrition, appear to apply similarly to poultry, where early growth enhances the deposition of omega-3s and antioxidants in eggs (Fleming et al., 2024).

Environmental conditions such as heat and rainfall significantly influence the nutrient composition of pasture-based systems. High summer temperatures accelerate plant senescence, leading to increased lignification and fiber content, while reducing concentrations of essential fatty acids and antioxidant compounds in forage (Bal & Minhas, 2017). In contrast, spring and early autumn rainfall promotes lush forage growth with a higher leaf-to-stem ratio, improving the availability of carotenoids and vitamins in pasture (Bist et al., 2024; Oke & Onagbesan, 2013). However, excessive moisture can result in leaching vital nutrients such as nitrogen and reduce

overall forage quality, potentially introducing microbial risks that impact both hen health and egg composition (Nardone & Valfrè, 1999).

In temperate regions, regrowth cycles further shape forage lipid profiles. ALA and total fatty acids tend to peak during early growth, decline during mid-season, and rise again with late-season regrowth (Chatzidimitriou, 2020). Optimizing grazing schedules and supplemental feeding can help maintain a consistent nutrient profile in eggs year-round. Seasonal nutrient shifts reinforce the importance of adaptive pasture management and dietary supplementation strategies to ensure consistent nutrient quality in pasture-raised eggs throughout the year.

### *1.6.2 Forage Composition and Nutrient Profiles Across Time*

The nutrient composition of forage varies significantly across plant species and evolves throughout the grazing season. Grasses, legumes, and forbs offer distinct profiles of crude protein, fiber, and fatty acids, directly influencing poultry diets and consequently, egg composition (Jaramillo et al., 2021). Plant maturity is a critical factor affecting digestibility and nutrient value; younger forages tend to have higher protein and lower fiber levels, making them more suitable for nutrient absorption (Spencer, 2013). As plants mature, lignin and fiber increase, reducing the bioavailability of essential nutrients.

Among forage species, legumes like alfalfa and clover provide rich sources of polyunsaturated fatty acids and protein, whereas grasses such as orchard grass and fescue contribute more structural carbohydrates but are lower in lipid content (Turner et al., 2014; Van Keuren & Matches, 1988). These differences highlight the importance of forage diversity and grazing timing to enhance the nutrient density of pasture-raised eggs throughout the production season.

### *1.6.3 Conserved Forages and Layer Hen Feeds*

When fresh pasture is unavailable, producers turn to conserved forages (e.g., hay, haylage, silage) and formulated layer hen feeds to support egg production and maintain nutrient quality. However, due to their monogastric digestive systems, hens are less able to extract nutrients from high-fiber forages compared to ruminants (Kutlu & Özen, 2009; Röhe & Zentek, 2021).

As a result, commercial layer diets rely on grains, oilseeds, fishmeal, and more recently, microalgae or flaxseed to enhance omega-3 content (Fraeye et al., 2012). These feed formulations can be specifically designed to boost omega-3 fatty acid deposition in egg yolks, a strategy not possible in beef systems where grain-feeding often reduces omega-3 levels in meat (H. Karsten et al., 2010; van Vliet, Provenza, et al., 2021). Studies have shown that supplementing hen diets with fish oil or algae leads to significantly higher levels of DHA and EPA in eggs, maintaining their health benefits even outside of grazing seasons (Fraeye et al., 2012) .

Understanding the species-specific nutritional demands of poultry versus ruminants is critical. Strategic supplementation ensures that even in the absence of fresh pasture, the nutritional quality of pasture-raised eggs can be preserved year-round.

## 1.7 Poultry Welfare and Environmental Considerations in Pasture Systems

### *1.7.1 Regenerative Agriculture and Pasture-Raised Systems*

Regenerative agriculture is an ecological farming approach focused on restoring soil health, enhancing biodiversity, and promoting self-sustaining agroecosystems. Techniques such as rotational grazing, cover cropping, and reduced chemical inputs improve carbon sequestration, water retention, and nutrient cycling, thereby supporting long-term ecosystem resilience and productivity (Atapattu et al., 2025; Krusinski, Sergin, et al., 2022).

Pasture-raised poultry systems align with regenerative principles by integrating hens into a biodiverse landscape. Rotational foraging prevents overgrazing and supports nutrient renewal in the soil. Moreover, Hens also assist with pest control and soil aeration while enriching soil through manure, enhancing microbial activity and fertility (Bilenky et al., 2024; Haschke et al., 2023).

By embedding poultry in regenerative systems, producers can simultaneously improve environmental outcomes and egg quality. However, maintaining pasture quality during seasonal transitions is a challenge, requiring adaptive management and nutritional supplementation (Caradus et al., 2024; Porras, 2024).

### *1.7.2 Production Scale and Economic Pressures*

Pasture-raised egg systems generally operate on a smaller production scale compared to industrial cage-based or cage-free systems. These systems require more land, labor, and management per bird, resulting in higher operational costs structural difference contributes to the premium price of pasture-raised eggs and limits their scalability in conventional supply chains (Meeh et al., 2014).

Recent market fluctuations, driven in part by the 2022–2023 outbreak of highly pathogenic avian influenza (HPAI), have further exposed vulnerabilities in the egg supply chain. Widespread culling of hens led to egg shortages and record-high prices in the United States (Caputo et al., 2023; Ufer, 2025). In this context, pasture-based systems offer potential value by supporting local food networks and distributing production risks. Localized pasture-based systems offer potential stability by supporting local networks and distributing production risks (Meyer et al., 2021; Watson, 2020).

### *1.7.3 Seasonal Variation and Environmental Uncertainty*

Despite their potential, pasture-raised systems face unique environmental challenges, particularly related to seasonal variation. Pasture quality fluctuates across the year due to temperature, precipitation, and plant maturity cycles, affecting the availability of key nutrients such as omega-3 fatty acids and carotenoids (Evaris et al., 2019). During non-growing seasons or extreme weather events, producers often struggle to maintain consistent egg nutrient profiles, especially when hens cannot access fresh forage (Cornell, 2020; Meeh et al., 2014).

This variability also raises questions about labeling accuracy and consumer expectations, especially as pasture-raised eggs grow in popularity. To date, little research has explored how specific climates or regions influence nutrient outcomes in pasture-raised systems.

### *1.7.4 Research Gaps and Objectives*

Although previous studies have consistently demonstrated the superior nutrient profiles of pasture-raised eggs compared to those from conventional systems, most research has evaluated these differences at isolated time points, without considering seasonal fluctuations in forage quality or environmental stressors (H. Karsten et al., 2010). Consequently, our understanding of how dynamic environmental and ecological conditions affect the nutrient composition of pasture-raised eggs remains limited.

A major gap lies in the lack of data on seasonal variation in forage-derived nutrients—such as carotenoids, omega-3 fatty acids, and antioxidants—and their relationship to the deposition of these compounds in egg yolks. While the responsiveness of egg nutrient profiles to hen diet is well-documented, fewer studies have examined this relationship across multiple months within a grazing season, particularly in the context of pasture-based systems that vary significantly in plant composition, climate, and management strategies (Fraeye et al., 2012).

Additionally, most existing literature has focused on nutrient enhancement through feed supplementation rather than environmental variability (Omri et al., 2019). This presents a further limitation, especially as pasture-raising systems grow in popularity as part of regenerative agriculture and local food economies, where nutrient inputs are more dependent on natural forage systems and less on processed feed (Krusinski, Maciel, et al., 2022). There is also a lack of data on how such seasonal nutrient variability may affect compliance with nutritional labeling standards or influence consumer health benefits.

Lastly, with the rising cost of eggs due to market disruptions like avian influenza, there is renewed interest in small-scale, local pasture-based production (Ufer, 2025). Yet, no current research thoroughly documents how seasonal nutrient variation in pasture-raised eggs occurs in specific regions, such as the Midwestern or Northeastern U.S., limiting the scalability and optimization of these systems

### 1.8 Conclusion

This chapter has reviewed the current scientific understanding of the nutritional composition of eggs and the many factors that influence yolk quality, including production system, hen diet, forage access, and environmental conditions. Numerous studies have shown that pasture-raised eggs are consistently higher in omega-3 fatty acids, carotenoids, and antioxidants compared to conventionally produced eggs, largely due to hen access to diverse, nutrient-dense forages (Fraeye et al., 2012; H. Karsten et al., 2010).

However, these nutrient advantages are not constant. The composition of forages—and therefore the nutrient intake of pasture-raised hens—varies throughout the grazing season due to plant maturity, species composition, temperature, and rainfall (Chatzidimitriou, 2020; Evaris et al., 2019). While some studies have explored the relationship between forage diversity and egg

nutrient enrichment, most have focused on dietary supplementation strategies and do not account for seasonal or regional variation (Omri et al., 2019).

Pasture-based systems, while nutritionally and ecologically promising, also face practical challenges. These include their smaller production scale, dependence on environmental conditions, and management limitations during non-grazing periods (Bilenky et al., 2024; Meeh et al., 2014). Yet, they also offer a resilient and localized alternative to large-scale egg production; especially in light of recent supply chain disruptions linked to avian flu outbreaks and rising egg prices (Meyer et al., 2021; Ufer, 2025). As demand for nutrient-dense and locally produced foods grows, understanding how to optimize egg quality within these systems becomes increasingly important.

Despite consistent findings that pasture-raised eggs are more nutrient-dense than conventionally produced eggs, the majority of existing studies capture nutrient data at only one point in time (Anderson, 2011; H. Karsten et al., 2010). Few have evaluated how seasonal variation in pasture quality—driven by changes in plant maturity, temperature, rainfall, and soil nutrients—impacts egg nutrition over time (Chatzidimitriou, 2020; Evaris et al., 2019).

While the effect of hen diet on egg nutrient content is well established, the role of environmental conditions and forage dynamics throughout a grazing season remains largely unexamined in poultry systems (Fraeye et al., 2012). This presents a critical research gap, particularly as regenerative, low-input production models gain traction (Atapattu et al., 2025).

Therefore, the purpose of the following study is to examine how seasonal changes in pasture conditions influence the nutritional composition of eggs in a pasture-based laying system. Through monthly sampling of forage, soil, and eggs, this study seeks to identify patterns in nutrient deposition across a full grazing season and assess how these variations align with changes in environmental and agricultural factors. This work builds on existing research in ruminant systems

and regenerative agriculture, and applies it to poultry systems: helping define optimal pasture-based practices for consistent and nutrient-dense egg production.

# CHAPTER II: GRAZING SEASON IMPACTS THE FATTY ACID AND NUTRIENT PROFILE OF EGGS ON A SOUTHERN OHIO PASTURE-RAISING SYSTEM FOR LAYER HENS

## 2.1 Abstract

Interest in regenerative poultry farming continues to grow, particularly due to its emphasis on soil conservation, biodiversity, and the natural interactions between hens and their surroundings. Access to pasture allows chickens to consume a diverse range of plants and insects, potentially enhancing the nutritional value of their eggs. However, nutrient composition fluctuates throughout the year as environmental conditions change. **Objective**: This study examined how seasonal changes in climate, soil composition, and forage availability influence the nutritional profile of eggs in a pasture-based laying system in Southern Ohio. Methods: Monthly collections of forage (n=3) and eggs (n=24, pooled into 12 replicates) occurred from May to December. Fatty acid composition was assessed using gas chromatography-mass spectrometry, while carotenoid and phenolic levels were measured colorimetrically. Vitamin and mineral content were analyzed through liquid chromatography and Inductively Coupled Plasma Optical Emission Spectroscopy. **Results:** Pasture quality, assessed by total digestible nutrients (TDN), peaked in October. Egg protein quality met USDA "Grade AA" standards every month except August ($p > 0.001$). The highest yolk pigmentation score was recorded in December ($9.5 \pm 1.3$; $p < 0.001$). Vitamin A levels were significantly greater in late summer ($p < 0.001$), while vitamin E gradually increased across the season, reaching its highest value in November ($118.1 \pm 24.0$ μg/g fresh yolk; $p < 0.001$). Carotenoid concentrations were elevated in mid-summer and late autumn ($p < 0.001$). Total omega-3 fatty acids were significantly higher in September and October than in mid-summer and late fall, while the n-6:n-3 ratio was lowest in early summer and fall compared to July ($p < 0.001$).

Principal component and random forest analyses demonstrated that eggs produced from September to November contained higher levels of vitamins A and E, greater essential omega-3 fatty acids, and a more favorable n-6:n-3 balance than eggs from other months. Conclusions: Significant seasonal shifts were observed in the fatty acid and antioxidant composition of pasture-raised eggs, with fall months yielding eggs with superior nutritional quality. These findings may assist consumers and producers in making informed decisions regarding the seasonal variation in pasture-raised egg nutrient composition.

## 2.2 Introduction

Pasture-raised egg farming has gained significant attention due to its emphasis on animal welfare, sustainability, and the production of nutrient-dense eggs, distinguishing it from conventional farming systems that rely heavily on confined animal operations and grain-based feed (H.D. Karsten et al., 2010). In a recent survey of U.S. consumers, 86% of respondents had purchased at least one animal product with welfare associated labels, such as “pasture-raised” (Thibault et al., 2022). This reflects a broader trend of prioritizing foods produced with improved environmental and ethical farming practices. Moreover, pasture-raised egg production is aligned with regenerative agricultural practices which focus on fostering a symbiotic relationship between chickens, forage, and the environment. These systems emphasize soil health, biodiversity, and ecological cycles while reducing the negative environmental impacts of conventional farming (Undersander D., 2014). Finally, hens in pasture-raising systems have access to a variety of plants and insects, contributing valuable nutrients that are otherwise absent or limited in conventional feed. Hens in these systems obtain nutrients directly from their environment, primarily through forage, making the nutrient composition of the pasture a critical determinant of egg quality and production.

Eggs from pasture-raised systems offer significant nutritional advantages over those produced in conventional systems, such as cage-free or caged systems, where hens are not required to have outdoor access (H.D. Karsten et al., 2010; Meng et al., 2014; Sergin et al., 2021; Sergin et al., 2022; Yenice et al., 2016). Pasture-raised eggs are generally more nutrient-dense compared to eggs from conventional caging systems, with one study reporting twice the vitamin E content and 2.5 times more omega-3 (n-3) fatty acids, contributing to a more favorable omega-6:omega-3 ratio (H.D. Karsten et al., 2010). The increased antioxidant, omega-3 fatty acid, and vitamin content in pasture-raised eggs is beneficial for human health, as consuming these nutrients supports immune function, reduces inflammatory cardiovascular diseases, and mitigates the harmful effects of oxidative stress (Jain et al., 2015; Réhault-Godbert et al., 2019; Simopoulos, 2008). These benefits are largely attributed to the inclusion of forages in the hens' diet, which have a higher antioxidant and polyunsaturated fat content—particularly n-3 fatty acids—compared to conventional corn- and soy-based feeds that are usually lower in antioxidants and higher in omega-6 (n-6) fatty acids. Moreover, the nutrient profile of eggs is highly responsive to dietary changes (H.D. Karsten et al., 2010; Krusinski, Maciel, et al., 2022).

Despite the benefits of pasture-based systems, seasonal variations in forage quality and composition significantly influence the nutrient profile of pasture-raised eggs. As plants mature, the leaf-to-stem ratio decreases, increasing acid detergent fiber (ADF) and neutral detergent fiber (NDF) while reducing digestible protein—essential for productive laying systems (Alex Rocateli, 2017). Lower total digestible nutrients (TDN), a key indicator of pasture quality, can negatively impact hens' growth, egg production, and nutrient absorption (Dillard, 2019). For example, alfalfa is associated with higher forage phenolic content, and orchard grass is linked to lower total forage carotenoids (Krusinski, Maciel, et al., 2022). Chickens preferences influence forage consumption,

where grass species, orchard grass and alfalfa have demonstrated highest palatability (Wood, 1956) Additionally, environmental factors such as rainfall, temperature, and soil conditions further influence forage quality (Alex Rocateli, 2017; Extension, 2023). Excessive rainfall can leach nutrients from plants, while prolonged high temperatures accelerate the degradation of fatty acids and antioxidants like vitamin E and carotenoids (Extension, 2023). This variability highlights the importance of understanding how seasonal changes in forage quality impact the nutrient profile of eggs.

While the effect of hen diet on egg nutrient composition is well documented, little research has focused on how seasonal variations in forage impact the nutrient profile of eggs in pasture-raised systems. Understanding these dynamics is crucial, as they could inform best practices for improving egg quality year-round in pasture-based systems. Therefore, the objective of this study was to document changes in the nutrient composition of forage and eggs in a Southern Ohio pasture-based system for layer hens across a grazing season. Further, we investigated how changes in egg nutrient composition connect to variations in weather, soil quality, and forage availability and composition and determined important discriminating factors in egg nutrient composition across the year.

## 2.3 Materials and Methods

### *2.3.1 Chemicals*

A gas chromatography–mass spectrometry (GC-MS) reference standard curve was created using the Supelco 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO, USA), along with individual standards, including mead acid, docosatetraenoic acid (DTA), n-3 docosapentaenoic acid (DPA), n-6 DPA, and palmitelaidic acid (Cayman Chemical, Ann Arbor, MI, USA). Branch chain fatty acids (BCFAs) were quantified using Mixture BR 3 (Larodan AB, Solna, Sweden),

while conjugated linoleic acid (CLA) isomers were quantified using the CLA reference standard UC-59M (Nu-Chek Prep, Elysian, MN, USA). Dichloromethane was obtained from VWR Chemicals (Radnor, PA, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise noted. All reagents used were HPLC grade unless otherwise noted, with isooctane being GC grade.

### *2.3.1 Diet Characteristics and Sample Collection*

This study was conducted across one grazing season (May-December 2022) at a privately managed farm in Southern Ohio (39.22°N, 84.34°W; 269 m elevation), where laying hens were rotated every 4 weeks between three 0.25 acre2 (1011.71 m2) fenced pastures. The farm was independently operated for production and not managed for research. Samples were collected during routine farm operations without experimental intervention or animal monitoring. As no animal handling or manipulation occurred, IACUC approval was not required. From May to September, the flock consisted of approximately 300 Comet hens; hens were around one year old at the start of collection. However, the flock size was drastically reduced by September due to predation and a high mortality rate. In response to these losses, Black Sex-linked hens, at an age of 16 weeks, were introduced in October, replacing the Comet hens. In rotation with grass-fed cattle, hens were rotated every 4 weeks across three fresh pastures. In addition, hens had free access to a standard layer hen feed all season (Table 1). The layer hen feed was sampled three times from a well-mixed bin of feed at the beginning and end of the grazing season each year for a total of $n$ = 6 replicates. Layer hen feed samples were freeze-dried and ground with dry ice to pass a 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and stored at −80 °C.

A total of 8 collections of forage, soil, eggs, and weather data were conducted from May to December, at 4-week intervals. Each month, before hens were given access to the pasture, forage

height and composition were assessed. Ten hoops (1/2 $m^2$) were randomly tossed across the pasture, and species percent coverage and pre-graze forage height were recorded from the center of each hoop. The same method was used to measure post-graze height after the hens were moved off the pasture, providing an estimate of forage intake across the month.

Then, when the hens were given access to the pasture, forage and soil samples were collected. To collect the forage, nine randomly selected 0.25 $m^2$ quadrats were clipped to a 1 cm stubble and thoroughly mixed. This process was repeated 3 times to create $n$ = 3 replicates of forage per month. Forage samples were promptly placed in a –20 °C freezer until delivery to the laboratory. Then, forage samples were freeze-dried and ground with dry ice to pass a 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and stored at -80 °C under nitrogen. At the same time, soil samples were collected. Using a soil probe, 15-20 subsamples were randomly taken in a zig-zag fashion from the pasture area and mixed in a bucket. This process was repeated 3 times to create $n$ = 3 replicates of soil per month.

After the hens had access to the pasture for several days, 36 eggs were randomly collected and, upon arrival at the laboratory, $n$ = 24 eggs were randomly chosen for analysis. Finally, weather data, including daily, monthly, and 30-year normal average temperature and total precipitation, was obtained from the U.S. Department of Commerce National Centers for Environmental Information (U.S. Department of Commerce, 2022).

**Table 1.** Composition of the layer hen feed

| Guaranteed Analysis | | Nutrition Requirement[1] |
|---|---|---|
| Crude Protein (Min) | 16.00% | 15.00% |
| Lysine (Min) | 0.85% | 0.69% |
| Methionine (Min) | 0.35% | 0.30% |
| Crude Fat (Min) | 3.50% | ND |
| Crude Fiber (Max) | 9.00% | ND |
| Calcium (Min) | 3.25% | 3.25% |
| Calcium (Max) | 3.75% | |
| Phosphorus (Min) | 0.70% | ND |
| Salt (Min) | 0.25% | 0.15% |
| Salt (Max) | 0.75% | |
| Selenium (Min) | 0.30 ppm | 0.60 ppm |
| Vitamin A (Min) | 882.00 IU/100 g | 3,000.00 IU/100 g |
| Vitamin D3 (Min) | 331.00 IU/100 g | 300.00 IU/100 g |

**Ingredients:** Wheat Midds, Oats, Barley, Organic Non-GMO Soybean Meal, Calcium Carbonate, Fish Meal, Kelp Meal, Salt, Monocalcium Phosphate, Brewers Grain Yeast, Lactobacillus acidophilus, Enterococcus faecium, Aspergillus oryzae, Bacillus subtilis, Bacillus licheniformis, Yucca schidigera, DL-Methionine, Vitamin A Supplement, Vitamin D3 Supplement, Vitamin E Supplement, Menadione Sodium Bisulfite Complex, Niacin, Riboflavin, D-Calcium Pantothenate, Pyridoxine Hydrochloride, Folic Acid, Zinc Amino Acid Chelate, Potassium Amino Acid Complex, Magnesium Amino Acid Chelate, Manganese Amino Acid Chelate, Copper Amino Acid Chelate, Vitamin B12 Supplement, Ferrous Sulfate, Manganese Oxide, Copper Sulfate, Sodium Selenite, Zinc Oxide, Choline Chloride, Ethylenediamine Dihydroiodide, Selenium Yeast.

[1]Represents layer hen intake requirements defined by the Nutrient Requirements of Poultry: Ninth Revised Edition, 1994 (Council). ND, not defined; IU, international unit.

### *2.3.3 Soil Analysis*

Soil samples were analyzed under the organic matter and general soil profile packages at a commercial laboratory provided through Michigan State University (East Lansing, MI, USA). Soil pH was assessed using a standard pH meter. Additionally, organic matter and ash content were determined using the loss on ignition (LOI) method using a muffle furnace. Mineral content was

assessed after the LOI ash product for Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) quantification.

### *2.3.4 Forage and Layer Hen Feed Proximate Analysis*

Forage and layer hen feed proximate analysis was conducted at the DairyOne Forage Laboratory in Ithaca, N, USA. Forage and feed moisture content was assessed using a forced air oven adapted from AOAC 991.01 and AOAC 930.15 methods, respectively (AOAC, 2023). Crude protein (CP), ADF, lignin, crude fat, and ash content were assessed using AOAC methods 990.03, 973.18, 973.18, and 954.02, respectively (AOAC, 2023). Forage and feed NDF content was assessed based on methods adapted from Van Soest et al (Van Soest et al., 1991). For the starch analysis, forage and feed samples were enzymatically digested into glucose using glucoamylase, then the resulting glucose was quantified indirectly using hydrogen peroxide equivalents measured by the YSI 2700 Select Biochemistry Analyzer. Metabolizable energy, digestible energy (DE), and TDN were calculated using the following equations (Council, 2001):

$$\mathrm{TDN_{1x}}(\%) = \mathrm{CP} + \text{ nonfiber carbohydrates} + (\text{crude fat} \cdot 2.25) + \mathrm{NDF} - 7$$

$$\mathrm{DE\ (Mcal/kg)} = \mathrm{TDN}(\%) \cdot 0.04409$$

$$\mathrm{ME\ (Mcal/kg)} = \mathrm{DE\ (Mcal/kg)} \cdot 1.01$$

### *2.3.5 Egg Physical Characteristics*

Egg physical characteristics were measured according as previously reported (Sergin et al., 2021; Sergin et al., 2022). Egg, yolk, and shell weight were recorded, and albumen weight was determined by subtraction. Albumen height was determined using a micrometer. Haugh units were determined from the recorded egg weight and albumen height (Haugh unit = 100 x log (albumen height (mm) + 7.57 – 1.7 x egg weight$^{0.37}$) (Eisen et al., 1962b). A colorimeter was used to quantify yolk color using the L*a*b (L* scale quantifies whiteness, a*, redness, and yellowness) (Spada et

al., 2016). Yolk color was also rated from 1 to 14 using the DSM yolk color fan (DSM Nutritional Products, Basel, Switzerland) (1 for pale yellow-16 for deep orange). Lastly, yolks were freeze-dried, powdered, and kept under nitrogen at -80 °C. Every two egg yolks were thoroughly mixed, creating n=12 replicates per month for subsequent analyses.

### *2.3.6 Fatty Acid Analysis*

Briefly, a modified version of the microwave-assisted extraction method by Bronkema et al (Bronkema et al., 2019) was used to extract fatty acids using 400 mg of egg yolk, forage, or layer hen feed samples and 8 mL of a 4:1 (v/v) ethyl acetate:methanol solution with 0.1% butylatedhydroxy toluene(BHT) as an antioxidant. Fatty acids were extracted in a CEM Mars 6 microwave (CEM Corp., Matthews, NC, USA) using the following microwave parameters: 55 °C for 15minuteswith an initial ramp of 2minutesat 400 W maximum power. Samples were then filtered and prepared as previously described to obtain the extracted oil (Sergin et al., 2021; Sergin et al., 2022).

Methylation described by Sergin et al (Sergin et al., 2021) modified from Jenkins (Jenkins, 1993) was conducted for the creation of fatty acid methyl esters (FAMEs). Two milligrams of extracted oil were combined with 500 µL toluene and 20 µg of methyl-12-tridecenoate (U-35M, Nu-Chek Prep, Elysian, MN, USA) as an internal standard. Base-catalyzed methylation was conducted using 2 mL of anhydrous potassium methoxide (0.5 N) at 50 °C for 10 min. Then, acid-catalyzed methylation was conducted using 3 mL of methanolic HCl (5%) at 80 °C for 10 min. Two mL of HPLC water were added, then FAMEs were extracted twice using 2 mL of hexane. Extracted FAMEs were resuspended in 1 mL of isooctane and stored at -20 °C until GC-MS analysis.

FAMEs were separated using the HP-88 column (100 m, 0.25 mm inner diameter, 0.2 µm film thickness; Agilent Technologies, Santa Clara, CA) on a Perkin Elmer 680/600 GC-MS (Waltham, MA, USA) in the electron impact (EI) mode with helium as the carrier gas (1 mL/min). For improved separation of fatty acid isomers, column temperature parameters described by Kramer et al. (Kramer et al., 2008) were used as follows: initial temperature of 80 °C for 4 min, ramp at a rate of 13.0 °C/min to 175 °C, held for 27 min, ramp at a rate of 4.0 °C/min to 215 °C, and held for 35minutes. Two different injections with a 1 µL injection volume and 250 °C injection temperature were conducted to capture both lower- and higher-concentration analytes. These were a 30:1 split injection and a splitless injection (0.75 minutes splitless hold time, 40 mL/min flow exiting the vent). Regarding MS settings, electron energy was 70 eV, and the transfer line and ion source temperature were set to 180 °C. MS data were recorded in full scan mode (m/z 70-400).

For identification of FAMEs, data were analyzed using MassLynx (4.1 SCN 714; Waters Corp., Milford, MA, USA). Retention time and EI mass fragmentation of each analyte were compared to those in our reference standard (described in section 2.1). Fatty acids not included in the reference standard were identified by elution order as reported by Kramer et al. (Kramer et al., 2008) and confirmed with EI mass fragmentation. Fatty acids were quantified using extracted ion chromatograms of the respective quantitative ions utilizing a standard curve constructed from our reference and internal standard. To calculate each FAME concentration, the internal standard peak area and analyte peak area in each sample were compared to those of the standard curve. Fatty acids were reported as percent of total fatty acids quantified and in g amounts per 100 g of egg yolk.

### *2.3.7 Phenolic Analysis*

Briefly, two extractions, first with 20 mL of a methanol:distilled water:acetic acid solvent [80:18:2 (v/v/v)], and second with 20 mL of an acetone:distilled water:acetic acid solvent [80:18:2 (v/v/v)], were used to extract phenolic compounds from 2 g of lyophilized egg yolk sample, ground forage, or ground layer hen feed. Tubes were shaken and centrifuged (840 *g*, 4 °C) and supernatants were combined following the addition of each solvent as previously described (Sergin et al., 2021; Sergin et al., 2022). Then, 100 µL Folin-Ciocalteu reagent and 800 µL 5% sodium bicarbonate were added to a gallic acid standard curve (1 mg/mL to 0.002 mg/mL) and to a 100 µL portion of the supernatant. These samples were heated at 40 °C for 30 min, cooled at room temperature for 10 min, and were plated in triplicate in a 96-well plate. Samples were then scanned in a microplate reader (Bio-Tek, Winooski, VT, USA) at 765 nm, compared against the standard curve, and reported as mg of gallic acid equivalents (GAE) per g of fresh egg yolk, forage, or feed.

### *2.3.8 Carotenoid Analysis*

For egg yolks, 0.5 g of lyophilized egg yolk sample was combined with 5 mL of cold acetone (0.05% BHT) and homogenized. Samples were vortexed for 2 min, then ultrasonicated in a water bath for 5 min, and centrifuged for 15minutes (1200 *g*, 4 °C). The supernatant was evaluated in a UV-Vis Double Beam Spectrophotometer (VWR, Radnor, PA, USA) at 450 nm against an acetone blank. Total carotenoid content was calculated according to Biehler et al (Biehler et al., 2010). using an ε of 140663 L/mol for beta-carotene in acetone and was expressed as µg of beta-carotene per g of fresh egg yolk.

For the forage and layer hen feed, in a conical tube, 2 g of ground sample were combined with 20 mL of 70% aqueous acetone. The tubes were shaken for 30minutesand centrifuged for 20minutesat 840 *g* and 4 °C. The supernatant was recovered in a new tube. The extraction was

repeated with an additional 20 mL of 70% aqueous acetone and the supernatants were pooled. Using the spectrophotometer, carotenoid and chlorophyll content of the supernatants were assessed in glass cuvettes at three wavelengths (663, 646, and 470 nm). Chlorophyll A, chlorophyll B, and total carotenoids were calculated using the following equations where $A_X = \text{Absorbance}_{X\,nm}$:

$$\text{Chlorophyll A } (C_a) = 11.75 \cdot A_{662} - 2.35 \cdot A_{645}$$

$$\text{Chlorophyll B } (C_b) = 18.61 \cdot A_{645} - 3.96 \cdot A_{662}$$

$$\text{Total Carotenoids} = \frac{1000 \cdot A_{470} - 2.27 * C_a - 81.4 \cdot C_b}{227}$$

### *2.3.9 Vitamin A and E Analysis*

Vitamin content of egg yolk, forage, and layer hen feed samples was assessed using the Veterinary Diagnostic Laboratory at Michigan State University (East Lansing, MI) using AOAC official method 2001.13 (AOAC, 2023). Briefly, lipid content was saponified using a potassium hydroxide solution in ethanol to reduce vitamin esters to their alcohol form. Vitamins were then extracted using hexane phase separation. Then, the extracted hexane layer was evaporated, and the residual vitamins were resuspended in acetonitrile:methylene chloride:methanol (70:20:10, v/v/v) for chromatographic analysis using an Acquity BEH C182, 1.7mm, 2.1 x 50 mm analytical column in a Waters Acuity system while using the Waters Empower Pro Chromatography Manger software. Vitamin quantification was assessed using the ApexTract method of Empower Pro using a calibration curve created using retinol, beta-carotene, and alpha-tocopherol standards (Sigma Aldrich, St. Louis, MO).

### *2.3.10 Mineral Analysis*

For egg yolks, 0.10 g of powdered yolk was predigested in borosilicate glass tubes with 3 mL of a concentrated ultrapure nitric and perchloric acid mixture (60:40 v/v) for 16 hours at room

temperature. Samples were then heated incrementally in a digestion block to 120 ºC for 4 h, followed by 2 h at 120 ºC with an additional 2 mL of nitric acid. The temperature was then increased to 145 ºC for 2 h and finally to 190 ºC to evaporate remaining liquid. Digested samples were resuspended in 10 mL ultrapure water and analyzed using Inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Thermo iCAP 6500 Series) with quality control standards for every 10 samples. Yttrium (0.50 µg/mL, final concentration) was added as an internal standard to ensure accuracy and correct for matrix interference.

For the forage and layer hen feed, 0.5 g of forage and layer hen feed samples were digested in 10 mL of a 4:1 (v/v) nitric:hydrochloric acid solution, followed by an additional 10-minute digestion with 1 mL of 30% hydrogen peroxide. Digestions were performed using a CEM Mars 6 microwave system (CEM Corp., Matthews, NC, USA) under the following parameters: a 10-minute ramp to 135 °C held for 3minutesat 1500 W, followed by a 12-min ramp to 200 °C held for 15minutesat 1600 W. Post-digestion, vessels were diluted to 50 mL, and aliquots were analyzed for mineral content using ICP-OES with a Thermo iCAP Pro XP radial spectrometer. For water analysis, 35 µL of concentrated nitric acid was added to 14 mL of water, mixed, and aspirated for ICP-OES measurement.

### *2.3.11 Egg Yolk Cholesterol Analysis*

Briefly, cholesterol was extracted from 0.5 g of freeze-dried powdered egg yolk by dilution using 9 mL of 2% (w/v) NaCl. Each replicate was vortexed for two minutes and shaken at 37 °C for 2 h. After solubilization, 0.5 mL of the solution was further diluted in 9.5 mL of 2% (w/v) and vortexed for 1 min. Then, the extraction solution was filtered through a 0.45 µm syringe filter to isolate cholesterol. 50 µl of the filtered, diluted, solution was calculated to contain 3-6 µg of

cholesterol. Quantification of the extracted cholesterol was determined colorimetrically following instructions using Cholesterol Quantification Assay kit (catalog: CS0005-1KT) produced by Sigma-Aldrich (Burlington, MA).

### *2.3.12 Statistical Analysis*

Means and standard deviations for each characteristic were calculated by month. To assess if egg yolk and forage nutrient content differed by month across the season, a one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test for significance was carried out using RStudio (R Core Team, Vienna, Austria). Results were considered significant at $p < 0.05$. Values under the limit of detection (LOD) were treated as zeroes. Additionally, a Spearman correlation analysis was carried out to explore how different factors were connected using the RStudio packages: ggplot2, reshape2, Hmisc, RColorBrewer, corrplot, showtext, readxl.

Further, MetaboAnalyst 5.0 (metaboanalsyt.ca) was used to carry out sparse partial least squares discriminant analysis (sPLS-DA) to visualize monthly groupings. Random forest (RF) analysis was to identify which nutrients were the strongest predictors for the separation of each month using 500 trees using OOB values with randomness (van Vliet, Bain, et al., 2021). Both analyses were conducted using yolk and forage antioxidants (total phenolics, total carotenoids, beta-carotene, vitamin A, and vitamin E) and fatty acids (% of total), and yolk cholesterol, with no data transformation or normalization necessary. Yolk mineral content was excluded from the sPLS-DA and RF analyses, as the minerals contributed minimally to the daily recommended intake for essential minerals, making them insignificant for this analysis.

## 2.4 Results

### *2.4.1 Weather*

The daily and monthly average temperature and total precipitation are shown in Figure 1. From May to December 2022 in Southern Ohio, daily temperatures followed expected seasonal patterns, increasing after May, peaking in July and August, and gradually decreasing as the season progressed. The monthly average temperature was closely aligned with the 30-year normal. The highest total precipitation was recorded in May and September. The monthly average precipitation differed from the 30-year normal throughout the season. The months of May, August, and September experienced higher total precipitation compared to the normal, while July and October had notably lower amounts.

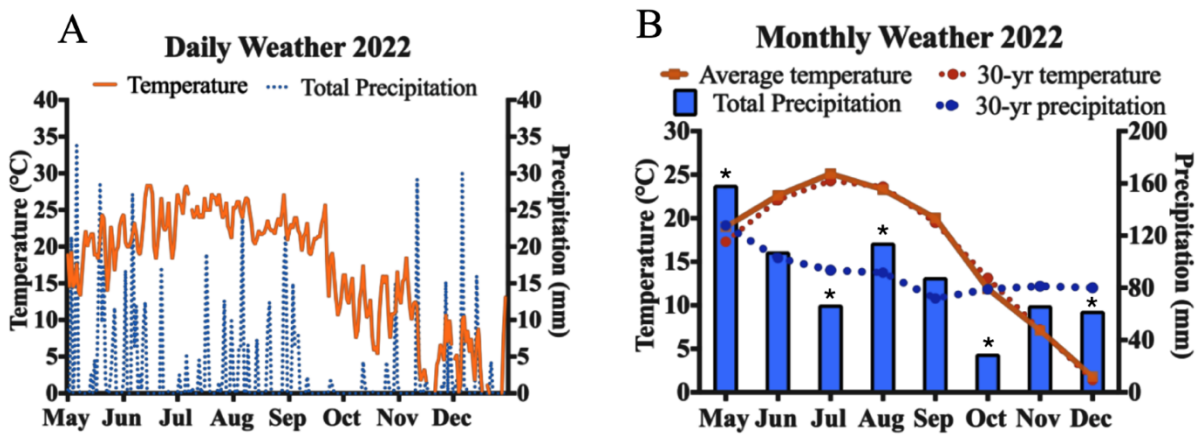


**Figure 1.** Weather trends across the 2022 grazing season. (A) Daily average temperatures and total precipitation (B) Monthly average temperature and total precipitation and their comparison to the 30-year normal. *Signifies average monthly total precipitation that is greater than three standard deviations from the 30-year normal.

### *2.4.2 Soil Composition*

Changes in the soil composition are shown in Table 2. Across the laying season, the soil pH and mineral content were sufficient to maintain forage quality (Kathrin Olson-Rutz, 2017). While several characteristics remained relatively stable across the season, such as pH, lime index, and organic matter, the mineral content fluctuated by month.

Additionally, several trends were observed when comparing soil characteristics with forage mineral content (**Appendix Table A1**). For example, phosphorus levels in the soil generally decreased over the season, dropping from 18.00 ppm in May to 5.00 ppm in August, before rising again in November ($p < 0.001$). The forage phosphorus levels followed a similar pattern, decreasing from 0.04% in May to 0.03% by November ($p = 0.035$).

**Table 2**. Characteristics of the soil by month[1]

| Parameter | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|
| pH | 6.40 ± 0.01 | 6.70 ± 0.26 | 6.77 ± 0.32 | 6.43 ± 0.32 | 6.57 ± 0.15 | 6.73 ± 0.21 | 6.53 ± 0.15 | 6.23 ± 0.06 | 0.088 |
| Lime index | 70.00 ± 0.01 c | 70.00 ± 0.01 c | 70.00 ± 0.01 c | 69.00 ± 0.01 d | 71.00 ± 0.01 b | 71.00 ± 0.01 b | 72.33 ± 0.58 a | 69.00 ± 0.01 d | <0.001 |
| Phosphorus (ppm) | 18.00 ± 4.36 bc | 13.67 ± 3.51 cd | 14.67 ± 1.53 cd | 5.00 ± 1.73 e | 7.00 ± 1.00 de | 13.33 ± 3.21 cd | 47.33 ± 3.51 a | 24.33 ± 1.15 b | <0.001 |
| Potassium (ppm) | 164.00 ± 30.51 b | 71.67 ± 18.50 b | 210.67 ± 51.19 ab | 100.33 ± 26.16 b | 97.00 ± 16.46 b | 197.33 ± 12.66 ab | 326.33 ± 121.71 a | 157.33 ± 34.67 b | <0.001 |
| Magnesium (ppm) | 228.33 ± 16.20 ab | 160.67 ± 7.77 c | 231.67 ± 13.58 a | 192.67 ± 13.05 abc | 195.33 ± 14.50 abc | 237.67 ± 35.57 a | 221.67 ± 6.35 ab | 181.67 ± 15.37 bc | <0.001 |
| Calcium (ppm) | 1413.33 ± 73.33 ab | 1591.33 ± 102.05 ab | 1706.67 ± 154.78 a | 1564.33 ± 249.5 ab | 1387.33 ± 93.11 ab | 1680.33 ± 178.21 a | 1552.00 ± 27.87 ab | 1237.67 ± 21.55 b | 0.008 |
| Cation exchange capacity (meq/100 g) | 9.40 ± 0.44 ab | 9.50 ± 0.52 ab | 11.00 ± 0.78 a | 10.50 ± 0.72 ab | 8.83 ± 0.55 b | 10.90 ± 1.23 a | 10.43 ± 0.42 ab | 9.33 ± 0.25 ab | 0.007 |
| | | | | % of Exchangeable bases | | | | | |
| % Potassium | 4.50 ± 0.98 ab | 1.97 ± 0.55 b | 5.00 ± 1.59 ab | 2.60 ± 0.30 ab | 2.87 ± 0.64 b | 4.67 ± 0.25 ab | 7.93 ± 2.63 a | 4.97 ± 0.93 ab | 0.001 |
| % Magnesium | 20.27 ± 0.50 a | 14.13 ± 0.58 c | 17.57 ± 0.21 ab | 16.73 ± 1.95 bc | 18.43 ± 0.81 ab | 18.13 ± 1.07 ab | 17.70 ± 0.98 ab | 18.33 ± 0.90 ab | <0.001 |
| % Calcium | 75.23 ± 0.55 cd | 83.90 ± 1.13 a | 77.47 ± 1.72 bcd | 80.63 ± 1.67 ab | 78.67 ± 0.99 bc | 77.20 ± 0.95 bcd | 74.33 ± 1.76 d | 76.40 ± 1.82 cd | <0.001 |
| Organic matter (%) | 4.47 ± 0.21 ab | 4.23 ± 0.35 ab | 4.23 ± 0.06 ab | 4.70 ± 0.20 a | 4.47 ± 0.06 ab | 4.67 ± 0.12 a | 4.13 ± 0.15 b | 4.03 ± 0.15 b | 0.003 |

[1]Means ± standard deviation (n = 3 soil samples per month) [2] Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ ($p < 0.05$). ppm, parts per million.

### *2.4.3 Forage Composition and Height*

The forage composition varied greatly across the laying season (Figure 2). The pasture featured a diverse mix of species, with the most prevalent being clover (*Trifolium repens*), fescue (*Festuca*), thistle (*Cirsium*), smartweed (*Persicaria lapathifolia*), and aster (*Tripolium pannonicum*). Additionally, the months with highest seasonal temperatures, July, and August, had the most plant diversity despite the impact of seasonal changes on the forage height. The difference between pre- and post-graze heights varied throughout the year. During peak summer, post-graze heights were especially low (e.g., June's drop from 59.1 cm to 6.2 cm). From September to December, forage consumption also shifted, leading to smaller differences between pre- and post-graze heights and a reduced variety of forage as the season was ending.

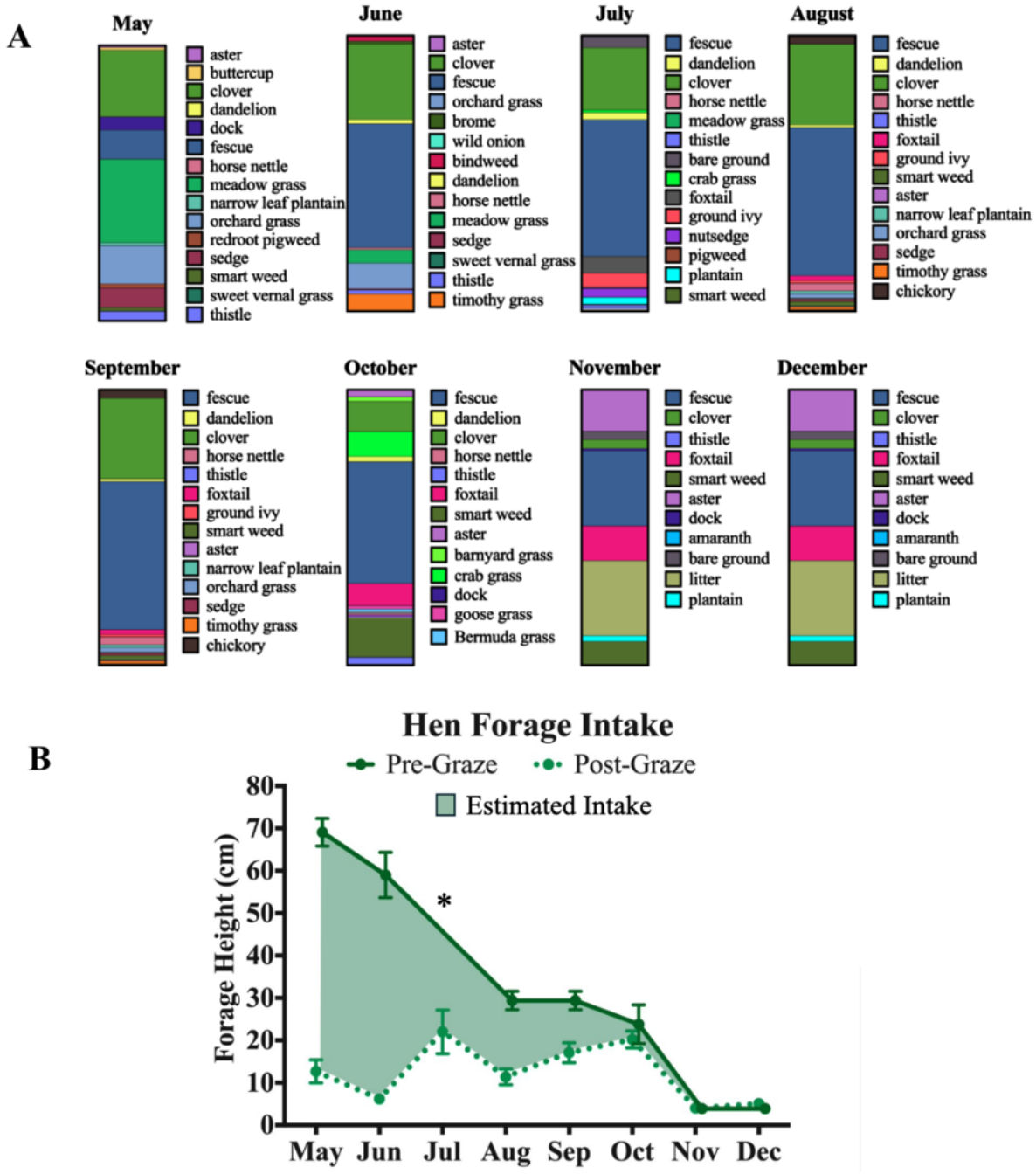


**Figure 2.** Monthly Forage Composition and Estimated Hen Forage Intake. (A) Illustrates the proportion and types of species that make up the monthly forage composition. (B) Height of forage before and after the hens grazed representing intake estimates across the year. *Pre-Graze height data not available

### *2.4.4 Forage and Layer Hen Feed Nutrient Composition*

Proximate analysis values for monthly forage and feed are displayed in Figure 3 and **Appendix Table A1**. In this study, the highest crude protein (CP) levels were observed in July (17.30 % DM) and October (17.40%), with lower levels in August (12.10 %) and December (12.47 %) *(p* = 0.003). Throughout the grazing season, ADF values ranged from 36% to 45% DM, with the lowest values in the early season and the highest in August, indicating increasing plant maturity by the end of summer *(p* < 0.001). Within this pasture raising system, TDN ranged widely across the grazing season from 47 to 61% DM *(p* < 0.018). Low quality forage TDN values fall within 45-52% DM, while mid quality forage ranges 52-58% DM, and high-quality forage exceeds 58% (Dillard, 2019) . Overall, the feed had a higher availability of digestible nutrients compared to the forage.

Additionally, forage and feed fatty acid profiles are presented in **Appendix Tables A2** and A3. Total forage fatty acids ranged from 3.625 g per 100 g in December to 14.801 g per 100 g in July (*p* = 0.121), whereas feed samples contained significantly more fat, averaging 164.131 g per 100 g of sample. Forage alpha-linolenic acid (ALA) content peaked in July at 6.681 g per 100 g and gradually decreased as the season progressed, reaching a low of 0.761 g per 100 g in December (*p* = 0.761). The feed samples contained higher total n-3 fatty acid levels (13.422 g per 100 g), nearly double the highest forage n-3 content (6.711 g per 100 g). Feed n-3 fatty acids primarily comprised docosahexaenoic acid (DHA) and DPA n-3. Forage and feed antioxidant data are detailed in **Appendix Table A4.** Forage carotenoid content was highest in May (765.92 µg per g) and lowest in November (73.06 µg per g) (*p* = 0.001) but remained significantly higher than feed carotenoid levels, which averaged 14.47 µg per g. Total forage phenolic content was generally higher than feed phenolic content, except in November.

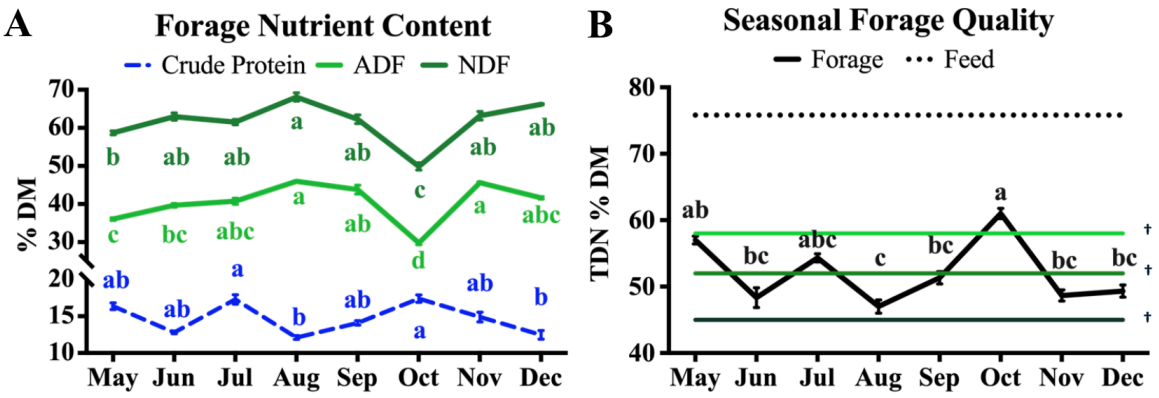


**Figure 3. Seasonal changes in the forage quality and proximate analysis.** Means and standard error of the mean (SEM) are shown. (A) Forage proximate analysis data (B) Forage quality based on total digestible nutrients. ADF; acid detergent fiber, NDF; neutral detergent fiber, TDN; total digestible nutrients. Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ $p < 0.05$. † Indicates Low (45%), Medium (52%), and High (58%) quality forage based on TDN (% DM)

### *2.4.5 Egg Characteristics*

Significant differences in the egg characteristics are shown in Table 3. Across the grazing season, significant differences were observed in egg weight ranging from 53 to 60 g *(p* = 0.004). Eggs from July and November were significantly larger compared to September *(p* = 0.004). The yolk fan values ranged from 7.08 in May to 9.54 in December *(p* < 0.001), with the highest value observed in the peak summer months and December. Based on colorimeter values, yolk colors were significantly lighter in June and September, had a more prominent yellow color in the month of October, and had strongest red influence in the month of August. Haugh units significantly varied, ranging from 61.20 in August to 88.28 in October *(p* < 0.001).

**Table 3**. Physical Characteristics of the Eggs by Month[1]

| Parameter | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|
| Egg weight (g) | 56.57 ± 5.43 ab | 58.16 ± 4.97 ab | 60.70 ± 6.73 a | 58.85 ± 9.93 ab | 53.39 ± 6.69 b | 57.73 ± 6.70 ab | 60.38 ± 4.94 a | 57.31 ± 4.24 ab | 0.004 |
| Shell weight (g) | 5.53 ± 0.54 abc | 5.73 ± 0.48 abc | 5.75 ± 0.84 abc | 5.91 ± 1.05 ab | 5.28 ± 0.79 c | 5.45 ± 0.82 bc | 5.92 ± 0.41 ab | 6.12 ± 0.49 a | 0.001 |
| Yolk weight (g) | 12.80 ± 1.00 cd | 13.00 ± 0.90 cd | 14.38 ± 2.14 ab | 14.02 ± 1.84 abc | 12.02 ± 1.88 d | 13.15 ± 1.65 bcd | 13.88 ± 1.22 abc | 14.73 ± 1.09 a | <0.001 |
| Dried yolk weight (g) | 6.58 ± 0.56 bc | 6.68 ± 0.51 abc | 7.32 ± 1.13 a | 6.92 ± 0.91 ab | 6.10 ± 0.99 c | 6.71 ± 0.93 abc | 7.11 ± 0.69 ab | 7.31 ± 0.61 a | <0.001 |
| Albumin weight (g) | 38.25 ± 4.79 ab | 39.42 ± 4.15 ab | 40.58 ± 5.08 a | 38.92 ± 7.66 ab | 36.10 ± 4.71 b | 39.13 ± 4.92 ab | 40.57 ± 3.99 a | 36.46 ± 3.26 ab | 0.010 |
| Albumin height (μm) | 7.28 ± 0.95 ab | 6.61 ± 1.04 bc | 5.85 ± 1.39 cd | 4.45 ± 1.21 e | 7.04 ± 1.44 ab | 7.73 ± 1.13 a | 6.63 ± 1.09 bc | 5.55 ± 0.99 d | <0.001 |
| Haugh unit | 86.21 ± 4.63 a | 81.27 ± 6.60 ab | 74.04 ± 12.01 b | 61.20 ± 18.34 c | 85.07 ± 9.43 a | 88.28 ± 6.51 a | 80.56 ± 7.65 ab | 73.81 ± 7.35 b | <0.001 |
| Yolk color fan[3] | 7.08 ± 1.59 d | 7.96 ± 1.20 bcd | 8.62 ± 1.41 abc | 9.00 ± 1.38 ab | 7.33 ± 1.88 cd | 8.38 ± 2.79 abcd | 8.79 ± 0.88 abc | 9.54 ± 1.38 a | <0.001 |
| Colorimeter[4] (L) | 67.55 ± 3.04 ab | 68.90 ± 1.86 a | 68.06 ± 2.52 ab | 66.71 ± 2.55 ab | 68.86 ± 2.98 a | 66.07 ± 3.94 b | 67.68 ± 1.21 ab | 65.78 ± 2.35 b | <0.001 |
| Colorimeter (a) | 10.68 ± 3.27 d | 14.83 ± 2.80 bc | 15.86 ± 3.29 abc | 19.26 ± 3.29 a | 14.58 ± 5.02 c | 17.74 ± 6.33 abc | 18.20 ± 1.56 ab | 17.34 ± 3.33 abc | <0.001 |
| Colorimeter (b) | 56.53 ± 4.02 d | 61.56 ± 2.94 bc | 60.64 ± 2.62 c | 64.60 ± 3.62 b | 60.57 ± 3.78 c | 69.83 ± 4.75 a | 61.46 ± 3.69 bc | 59.52 ± 4.10 cd | <0.001 |

[1] Means ± standard deviation ($n$ = 24 eggs per month) [2] Results of one-way ANOVA. [3]Yolk color fan was measured on a scale of 1-16 from light yellow to dark orange. a-e, Means within a row with different letters significantly differ ($p < 0.05$). [4] Colorimeter numerically assess color gradient (L* scale quantifies whiteness, a*, redness, and b*, yellowness)

### *2.4.6 Egg Yolk Antioxidants*

Changes in the yolk antioxidant profile can be observed in Figure 4 and **Appendix Table A5.** Significant changes in the yolk antioxidant profile were observed across the season based on vitamin total carotenoids, beta-carotene, and vitamin E content. Total yolk phenolic content

remained stable across the year, showing no apparent seasonal variations. ($p = 0.019$). Vitamin A levels in egg yolks gradually increased throughout the summer, peaking in September ($p < 0.001$), while Vitamin E rose significantly from May to November before dropping sharply in December ($p < 0.001$). Conversely, total carotenoid levels rose from May, peaking in August, and remained relatively high through October before stabilizing in December ($p < 0.001$), with similar trends observed with beta-carotene content.

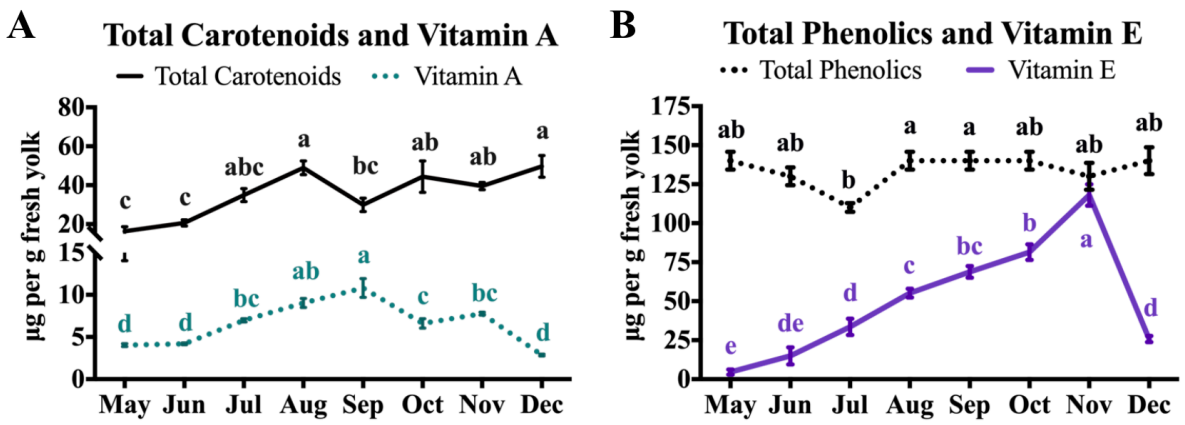


**Figure 4. Significant changes in the yolk antioxidant profile.** Monthly means and SEM are shown. (A) Changes in the vitamin A and total carotenoid content (B) Changes in yolk vitamin E content. Results of one-way ANOVA. a-e, means within a row with different letters significantly differ ($p < 0.05$).

### *2.4.7 Egg Yolk Fatty Acid Profiles*

Seasonal variations in yolk fatty acids are presented in Figure 5, Table 4 and **Appendix A6 and A7**. Significant monthly changes in fatty acid profiles were observed throughout the grazing season, with total fatty acids peaking in May at 20.84 g per 100 g and progressively declining to a low of 11.38 g per 100 g in October, before continuing to decrease through December. These values remained consistently below the expected 28.8 g per 100 g of fresh yolk ($p < 0.001$). Saturated fatty acids were significantly lower than expected USDA values, with total palmitic acid peaking at 4.98 g per 100 g ($p < 0.001$) compared to the expected 6.8 g per 100 g, while total

stearic acid content consistently fell below the USDA expectation of 2.42 g per 100 g of yolk ($p < 0.001$) (Agriculture, 2019). Across the season, cholesterol ranged from 0.809 g in May to 1.209 g per 100g in September *(*$p < 0.001$) peaking halfway through the season. Although slight variations were observed in cholesterol content, overall, the content was close to the expected USDA value of 1.08 g per 100 g of egg yolk (Agriculture, 2019).

Omega-3 content varied widely throughout the grazing season, with lower levels (0.234 g to 0.516 g per 100g) observed during the late spring and summer months, followed by a significant increase to 1.349 g per 100 g in September ($p < 0.001$). The n-6:n-3 ratio was closest to the recommended 4:1 during the fall months ($p < 0.001$). As shown in **Appendix Table 3**, relative n-6 content exhibited only minor fluctuations across the season, indicating that the lower ratio observed in the fall was primarily driven by the substantial increase in n-3 fatty acids rather than changes in n-6 levels. Changes across the grazing season were observed in the branched-chain (BCFA) and conjugated linoleic acid (CLA) fatty acids in Table 3 and **Appendix Table A7**. Branch chain fatty acids that were quantified in this pasture-raised system were C15:0-*iso,* C15:0-*anteiso*, C16:0-*iso*, C17:0-*iso*, C17:0-*anteiso*, C18:0-*iso*, and C18:0-*anteiso*. Total BCFA levels were significantly higher in September compared to May, July, August, October, and December, indicating a seasonal effect on BCFA production *(*$p = 0.002$). In this system, CLA was present in the egg yolks in four isomers: cis-9, trans-11, trans-10, cis-13, and trans-trans CLA. Total CLA ranged from 0.21% in May to 0.41% in October *(*$p < 0.001$) (Mir et al., 2004).

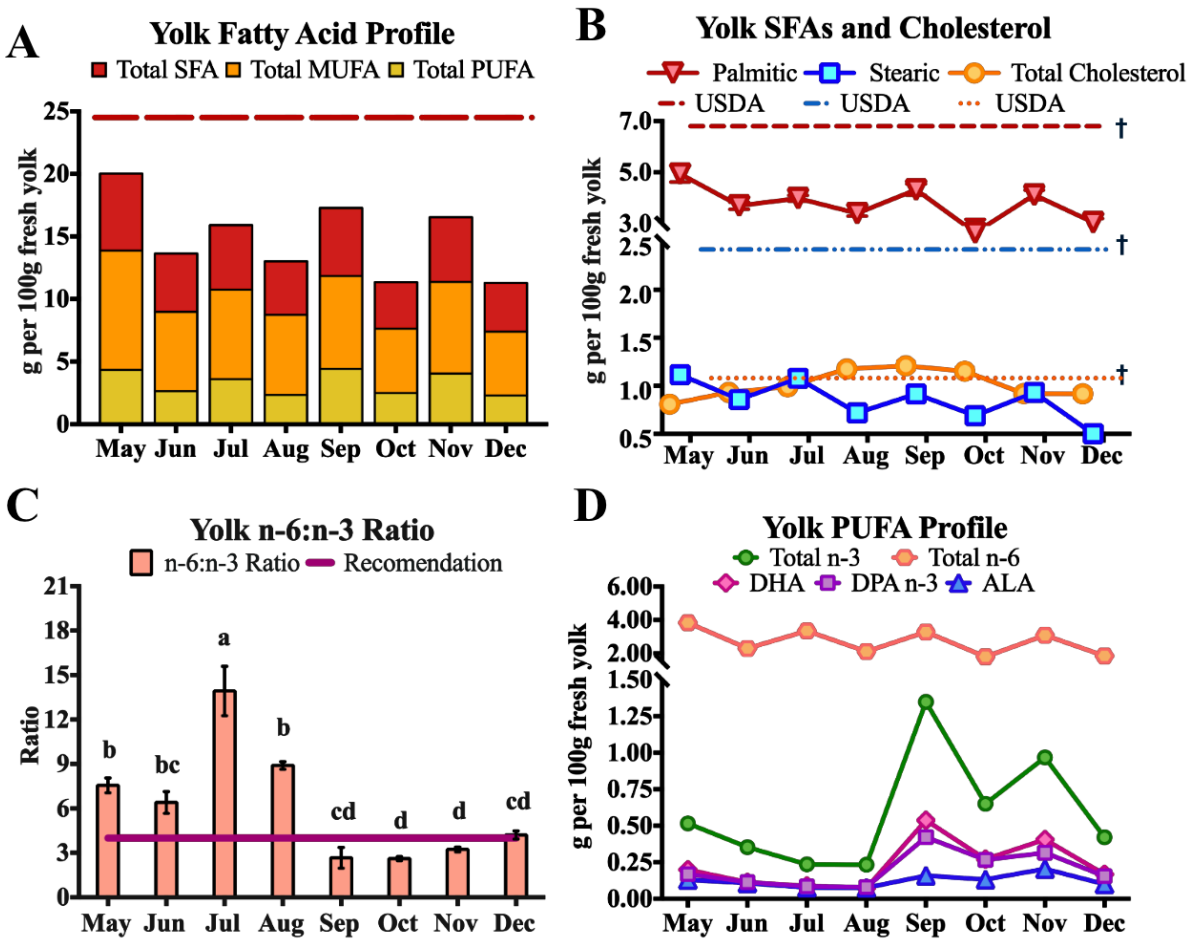


**Figure 5. Notable Seasonal Variations in the Yolk FA Profile.** Monthly means and SEM are shown. (A) Total SFA, MUFA, and PUFA values across the grazing season compared to the USDA expected value for total FA. (B) Palmitic, stearic, and total cholesterol across the season compared to expected USDA nutrient content. (C) Monthly changes in the n-6:n-3 ratio compared recommendation (Simopoulos, 2008). (D) Seasonal variations in the total and individual omega-3 fatty acid content. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; † USDA Cage-Free Egg Yolk expected nutrient value (25.45 g Total Fat, 6.86 g Palmitic Acid, 0.104 g Stearic Acid, and 1.08 g per 100 g)(Agriculture, 2019). Results for the Yolk SFAs, cholesterol, and the n-6:n-3 ratio as shown as mean ± SEM.

**Table 4.** Egg yolk branched chain and conjugated linoleic fatty acids by month (g of fatty acid per 100 g of fresh egg yolk)[1]

| Fatty Acid | Carbon Number | May | Jul | Sep | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|
| CLA | 9c, 11t 18:2 | 0.016 ± 0.002 b | 0.012 ± 0.002 cd | 0.022 ± 0.006 a | 0.017 ± 0.003 b | 0.022 ± 0.002 a | 0.015 ± 0.002 bc | <0.001 |
| | 11t, 13c 18:2 | 0.009 ± 0.001 c | 0.009 ± 0.001 bc | 0.012 ± 0.002 a | 0.01 ± 0.001 c | 0.011 ± 0.001 b | 0.010 ± 0.001 bc | <0.001 |
| | 11t, 13t 18:2 | 0.032 ± 0.002 bc | 0.026 ± 0.004 de | 0.046 ± 0.007 a | 0.033 ± 0.005 b | 0.041 ± 0.003 a | 0.031 ± 0.004 bcd | <0.001 |
| | t, t 18:2 | 0.009 ± 0.001 b | 0.010 ± 0.001 b | 0.011 ± 0.002 a | 0.009 ± 0.001 b | 0.010 ± 0.001 b | 0.009 ± 0.001 b | <0.001 |
| C14:0-*iso* | 14:0 | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| C15:0-*iso* | 15:0 | 6.812 ± 1.209 a | 5.548 ± 0.539 b | 6.591 ± 1.447 ab | 4.206 ± 1.158 c | 6.238 ± 0.677 ab | 4.194 ± 0.645 c | <0.001 |
| C15:0-*anteiso* | 15:0 | 9.667 ± 2.024 a | 7.382 ± 0.818 b | 7.26 ± 1.039 b | 5.139 ± 1.593 c | 7.311 ± 0.783 b | 5.307 ± 0.881 c | <0.001 |
| C16:0-*iso* | 16:0 | 3.684 ± 0.957 a | 3.271 ± 0.983 ab | 2.925 ± 0.649 abc | 1.675 ± 0.661 e | 2.907 ± 0.495 abc | 1.760 ± 0.318 de | <0.001 |
| C17:0-*iso* | 17:0 | 3.290 ± 0.899 a | 2.996 ± 0.975 ab | 2.629 ± 0.588 abc | 1.457 ± 0.560 e | 2.565 ± 0.45 a bc | 1.588 ± 0.283 de | <0.001 |
| C17:0-*anteiso* | 17:0 | 0.317 ± 0.072 a | 0.215 ± 0.053 bcd | 0.243 ± 0.067 abc | 0.184 ± 0.101 cd | 0.292 ± 0.059 ab | 0.147 ± 0.042 d | <0.001 |
| C18:0-*iso* | 18:0 | 10.596 ± 2.424 b | 14.575 ± 5.416 a | 11.161 ± 2.014 ab | 8.680 ± 2.516 b | 8.934 ± 1.500 b | 11.291 ± 2.691 ab | 0.006 |
| C18:0-*anteiso* | 18:0 | 0.083 ± 0.022 a | 0.063 ± 0.015 bc | 0.067 ± 0.014 ab | 0.034 ± 0.015 d | 0.058 ± 0.017 bc | 0.034 ± 0.006 d | <0.001 |
| Total CLA | | 0.066 ± 0.005 cd | 0.057 ± 0.007 cd | 0.083 ± 0.027 a | 0.069 ± 0.010 bc | 0.083 ± 0.006 ab | 0.065 ± 0.008 cd | <0.001 |
| Total BCFA | | 0.071 ± 0.005 b | 0.071 ± 0.007 b | 0.084 ± 0.014 a | 0.072 ± 0.008 b | 0.075 ± 0.004 ab | 0.072 ± 0.007 b | <0.001 |
| Total *iso*BCFA | | 0.055 ± 0.004 b | 0.055 ± 0.006 b | 0.065 ± 0.013 a | 0.056 ± 0.006 ab | 0.058 ± 0.003 ab | 0.057 ± 0.006 ab | 0.001 |
| Total *anteiso*BCFA | | 0.016 ± 0.001 bc | 0.016 ± 0.002 bc | 0.019 ± 0.002 a | 0.015 ± 0.002 c | 0.016 ± 0.001 bc | 0.015 ± 0.002 bc | <0.001 |

[1] Means ± standard deviation (n = 24 eggs pooled into n = 12 replicates per month) [2] Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ ($p < 0.05$). OCFA, odd-chain fatty acids; CLA, conjugated linoleic acid; FA, fatty acids.

### *2.4.8 Yolk Mineral Profile*

The yolk mineral profile is reported in **Appendix Table A8**. Seasonal changes in the yolk mineral profile were observed, with phosphorus, magnesium, and manganese generally peaking during the summer months, particularly in July ($p < 0.001$ for all). In contrast, sodium levels were notably higher in November and December compared to the rest of the grazing season ($p < 0.001$). Essential minerals, including calcium, potassium, magnesium, iron, zinc, and selenium, were insufficient throughout the season for the average yolk to be classified as a high source of nutrients (Health, 2024).

### *2.4.9 Correlations between yolk and forage nutrients and seasonal impacts*

In Figure 6, Spearman correlations were carried out across yolk nutrients, forage nutrients, individual forage species, and environmental changes to demonstrate significant relationships across the whole biosystem ($p < 0.05$). Yolk cholesterol content observed strong positive relationships with orchard grass, fescue, and horse nettle forage species. Yolk total carotenoid and beta-carotene content were significantly associated with the month, forage vitamin E, meadow grass, foxtail species. Rainfall displays a strong negative relationship with forage vitamin E. The yolk fan score was primarily linked to forage nutrient parameters but unexpectedly showed an inverse relationship with forage phenolics and total carotenoid content. Additionally, among the yolk nutrients most influenced by forage intake, no significant relationships were observed between their levels in the forage (n-3 PUFAs, carotenoids, vitamin E, phenolics) and their corresponding levels in the eggs.

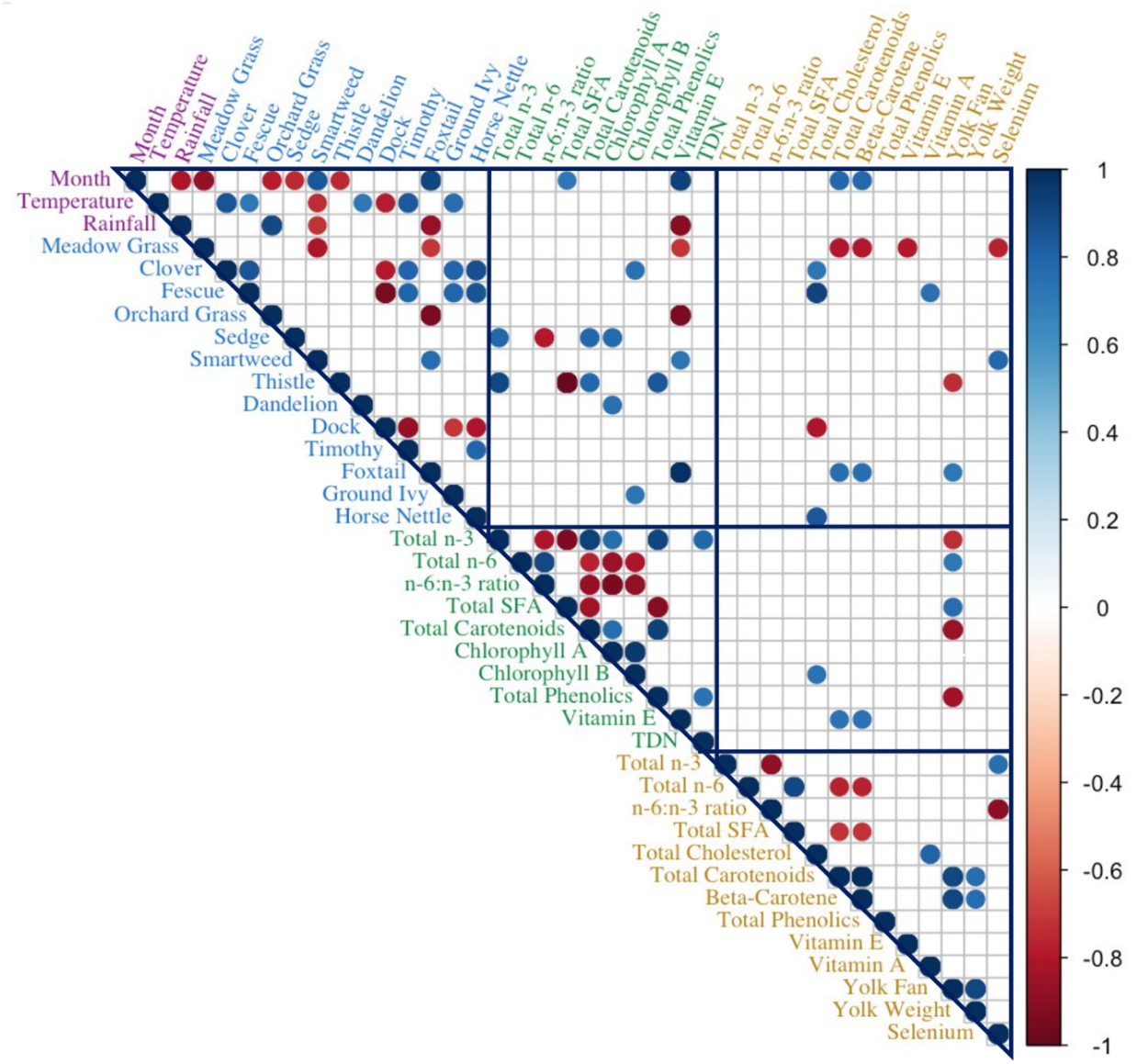


**Figure 6.** Spearman correlation matrix illustrating significant relationships across monthly averages of egg nutrients, forage nutrients, environmental changes, and forage species parameters ($p<0.05$). The color intensity represents the strength of the correlation depicted: Blue represents R coefficient values between 0 to 1, while red represents values between 0 to –1. Text colors distinguish between sample type: purple for environment, blue for forage species present in the pasture, green is assigned to forage nutrients, and yellow to egg nutrients. total omega-3 fatty acids, total n-6; total omega-6 fatty acids, total SFA; total saturated fatty acids; TDN, total digestible nutrients

### *2.4.10 Yolk, Forage, and Feed Discriminant and Random Forest Analysis*

The sPLS-DA and random forest analysis results are presented in Figure 7 with yolk and forage PCA loadings displayed in **Appendix Tables A9 and A10**, respectively. (A) The sPLS-DA plot of yolk nutrients shows minimal separation between May to August and December, with noticeable differentiation observed during the fall months of September through November. (B) The forage and feed sPLS-DA scores plot indicate consistent overlap in forage nutrient profiles across all months, while feed nutrients exhibit distinct separation. (C) The random forest analysis highlights the importance of yolk nutrients in distinguishing individual months. Vitamin A and E emerged as the most discriminative variables, followed by DHA (C22:6 n-3), total n-3 fatty acids, and the n-6:n-3 ratio. These findings further confirm the separation of September through November from other seasons, driven by a lower n-6:n-3 ratio, higher levels of essential n-3 fatty acids, and elevated vitamin E and A concentrations during the fall months. (D) The forage and feed random forest analysis identified saturated fat and vitamin E as the most critical indicators of separation. The feed samples were characterized by a lower saturated fat profile and reduced vitamin E levels compared to forage. Vitamin E content was highest in the forage in December (**Appendix Table A10**), meanwhile vitamin E was lowest in the eggs during the same month.

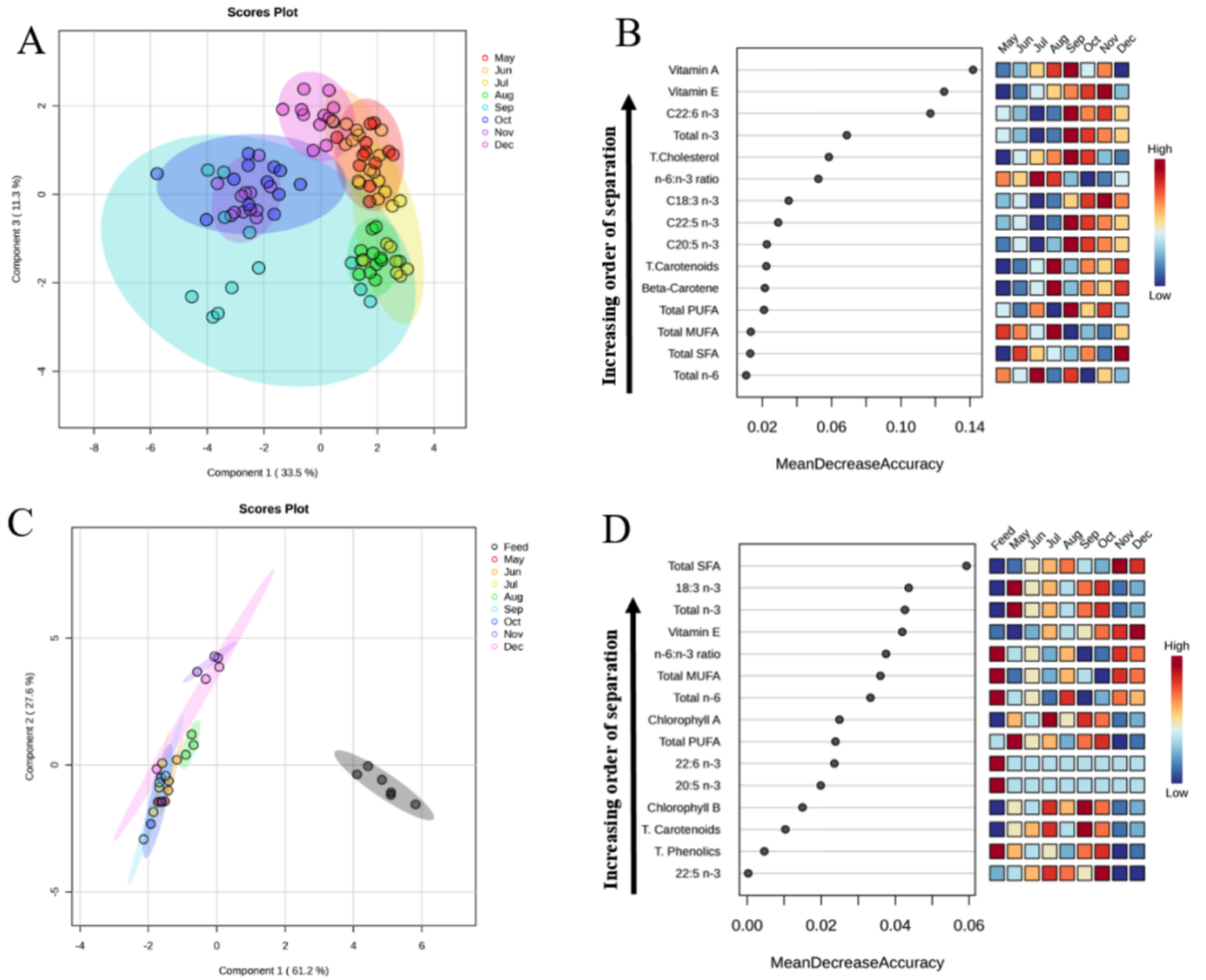


**Figure 7. System Nutrient Structure**. (A) sparse Partial Least Squares Discriminant Analysis (sPLS-DA) plot using egg nutrient parameters only showing separation and clusters based on month, with some overlaps. (B) Random Forest (RF) variable importance plot showing yolk nutrient parameters that differentiate between monthly collections. (C) sPLS-DA plot using forage and feed nutrient parameters only showing separation and clusters based on month, with some overlaps. (D) RF variable importance plot showing nutrient parameters that differentiate between monthly collections and layer hen feed. For sPLS-DA plots, elipses are representative of 95% confidence interval regions. For RF plots, the y-axis represents nutrient parameters in order of importance for monthly classification (from top to bottom). The x-axis shows mean decrease accuracy, with a higher value indicating the importance of that phytochemical in predicting groups. Total SFA; total saturated fatty acids, total MUFA; total monounsaturated fatty acids, total PUFA; total polyunsaturated fatty acids, t. carotenoids; total carotenoids, total n-3; total omega-3 fatty acids, total n-6; total omega-6 fatty acids, t. phenolics; total phenolics.

## 2.5 Discussion

In the present study, we demonstrated significant changes in the nutrient profile of eggs including the fatty acid and antioxidant composition that related to seasonal fluctuations in weather, soil quality, and forage composition. The findings of this study highlight the interwoven nature of environmental factors and egg nutrient quality within free-living animal production systems.

Pasture-raising systems are shaped by numerous external influences on chicken habitats, identifying factors that impact dietary preferences or the prediction of yolk nutrient deposition is challenging, emphasizing the need for this research. High forage consumption is typically expected to increase yolk levels of vitamin E, omega-3 PUFAs, carotenoids, and phenolics, as observed in grass-fed versus grain-fed systems (Krusinski et al., 2023; van Vliet, Bain, et al., 2021). However, pasture-raising systems are shaped by numerous external influences on chicken habitats and dietary patterns. Chickens are considered omnivores and cannot thrive on a forage-only diet; they prefer insect-based diets and are often consuming rocks and ground material to aid in their digestion (Belhadj Slimen et al., 2023; Kilpatrick, 2022). Their opportunistic feeding habits make it difficult to accurately measure their intake while maintaining their pastured lifestyle, which may contribute to seasonal variations in yolk nutrient profiles.

Seasonal variations in the yolk fatty acid and mineral profile were likely due to diminished forage consumption and greater reliance on supplemental feed. Most notably, the n-6: n-3 ratio fluctuated across the season, ranging from 2.78 to 13.72. Eggs collected during the fall months (September–December) achieved the recommended 4:1 ratio, driven primarily by the increased n-3 content, as linoleic levels remained relatively stable (Simopoulos, 2008). Yolk nutrient profiles during the fall particularly reflected the influence of feed, with random forest analysis revealing that total n-3 and DHA levels in yolks did not align with the seasonal high and low omega-3 density

observed in forage. Instead, yolks produced between September and November contained significantly higher amounts of n-3 PUFAs, vitamin E, and vitamin A, accompanied by the lowest n-6:n-3 ratio. These improvements were primarily attributed to increased feed consumption, as feed was heavily supplemented with omega-3 fatty acids and vitamin A. This disconnect between forage nutrient density and yolk deposition could reflect a combination of factors including low actual forage intake, selective foraging behavior, nutrient loss during digestion, or differential metabolic prioritization of nutrients under varying environmental conditions.

Yolk CLA and BCFAs, showed seasonal patterns influenced by diet, with CLA synthesis linked to linoleic acid levels (Mir et al., 2004; Nasrollahzadeh et al., 2023; Qaisrani et al., 2015). Exposure to cattle in regenerative systems may account for the presence of uncommon BCFAs, such as C18:0-iso, which are typically found in cattle products and rarely detected in eggs (Patel et al., 2013; Ran-Ressler et al., 2014; Sergin et al., 2021; Sergin et al., 2022; Undersander D., 2014). Mineral content was relatively stable throughout the season, except for potassium, which spiked in the fall months. This increase likely reflects the layer hens' greater reliance on feed rather than fresh forage during the colder months, as the feed contained a higher sodium concentration compared to forage. Additionally, reliance on supplemental feed is further supported by the sPLS-DA scores plot for yolk nutrients, which showed fall months clustering separately from earlier months and December, Similarly, forage and feed sPLS-DA plots demonstrated distinct groupings, suggesting feed became the primary driver of yolk nutrient changes during the fall. Additionally, weather deviations from seasonal norms—such as heavier or lighter rainfall observed in this system—may have further influenced forage quality and availability, thereby affecting hen foraging behavior and feeding preferences (Vallentine, 2000). This pattern underscores the critical

role of feed supplementation in meeting the nutritional needs of pasture-raised hens when environmental conditions limit forage availability.

Antioxidant deposition in egg yolks reflected the interplay between forage nutrient content and environmental stress. Vitamin E levels in yolks closely mirrored forage vitamin E levels throughout most of the season, except in December, when cold stress likely redirected vitamin E toward the hens' metabolic needs rather than yolk deposition (Kim et al., 2023). In addition to cold stress, predator disturbances may have also contributed to behavioral or metabolic changes, further affecting nutrient deposition in the eggs. Vitamin A content peaked gradually from September to December. This discrepancy may be attributed to cold stress (<16°C), as dropping temperatures prompt hens to prioritize heat generation over digestion and nutrient absorption, resulting in lower vitamin levels in eggs (Kim et al., 2023; Sahin et al., 2003). Carotenoid levels in yolks, however, remained stable even during colder months, showcasing the hens' ability to maintain antioxidant deposition despite environmental challenges. It is also possible that differences in nutrient bioavailability and deposition efficiency, particularly under stress conditions, influenced which forage-derived compounds were absorbed and stored in yolk tissue. These findings highlight the importance of forage-derived antioxidants, particularly during the summer and early fall.

Yolk color, a key quality parameter influenced by carotenoid content, fell short of consumer preferences despite seasonal variations. Consumer preferences align with the darkest values of the DSM Yolk Color Fan, which are associated with more nutrient-rich yolks (Hernandez et al., 2005). However, yolks in this system were far below consumer preferences, while also being lower than some cage-free eggs, which were given an average DSM value of 10.3 in a similar study (Bertoncelj et al., 2019; Kojima et al., 2022; Sergin et al., 2021). While differences in yolk pigmentation were observed, the reason for these changes is unclear. The introduction of Black

Sex-linked hens in October, replacing Comet hens, did not impact the overall trend in yolk color. Previous research has shown that while diet is the primary factor influencing yolk carotenoid content, breed may also play a role in carotenoid absorption and metabolism, potentially contributing to differences in yolk pigmentation (Kojima et al., 2022). This suggests that both forage composition and breed-specific factors could influence yolk pigmentation and should be further explored in future studies.

Additionally, seasonal changes in egg white protein quality, measured by Haugh units (HU), were closely tied to temperature fluctuations**.** Eggs produced in this system overall met the USDA AA quality standard (HU ≥ 72), except in August, when values dropped to Grade A (Eisen et al., 1962a; Gabriela da Silva Pires et al., 2020). Lower HU values were observed during the hot summer (July–August) and cold winter (November–December) months, aligning with environmental stressors outside the optimal laying temperature range of 19–22 °C (Pawar et al., 2016). The highest HU values occurred in May and September, when temperatures were within the thermoneutral zone, highlighting the role of temperature in albumen quality and freshness (Barrett et al., 2019). Based on our results, the majority of eggs met the necessary criteria for sale under U.S. food laws. The only notable exception was albumen quality in August, where Haugh unit values fell into the USDA Grade A range rather than AA. While seasonal fluctuations in omega-3 levels were observed, these variations did not appear to impact regulatory compliance. However, such fluctuations could influence nutrient labeling or marketing claims related to omega-3 content. Further research could assess whether seasonal shifts impact classification for commercial sale.

A key limitation of free-living systems is the difficulty in controlling hen intake while adhering to pasture-raising principles, especially regarding non-pasture ingredients such as insects.

Free-living systems carry the difficulty in controlling hen intake while adhering to pasture-raising principles, particularly regarding non-pasture ingredients such as insects**.** Several insect species known to be nutrient-rich—black soldier fly larvae, crickets, mealworms, house flies, and maggots—are commonly found in Southern Ohio, particularly during the warmer months (May–September) when pasture-raised hens are actively foraging. These insects are known to contain measurable amounts of omega-3 fatty acids and can also be rich in vitamin E, and carotenoids, though their nutritional profiles vary depending on life stage and diet (Kolobe et al., 2023; Schiavone et al., 2019). While insect intake was not directly measured in this study, previous research suggests that for-age—including insects, worms, and plants—contributes approximately 5–10% of the hens' diet in pasture-based systems (Schiavone et al., 2019). Insects likely comprise only a subset of that total, but given their nutrient density, even small amounts may meaningfully influence egg nutrient composition. The seasonal presence of these insects may help explain some of the trends in omega-3 and antioxidant levels observed in the eggs and points to an important area for future investigation.

Practical challenges like predation led to the introduction of younger Black Sex-linked hens in October, replacing Comet hens and is a limitation of this study. We acknowledge that the breed change was a significant alteration in the study population and may have contributed to observed changes in egg nutrient profiles. While both breeds were managed identically and had access to the same diet, differences in nutrient metabolism, or age-related physiology could not be separated from seasonal effects, as no overlap in time points existed between the two breeds. Breed differences are generally associated more with eggshell color and production rate than with yolk nutrient profiles (Drabik et al., 2021). In addition, younger hens may exhibit different nutrient deposition patterns during their peak laying period, potentially affecting yolk composition

independent of diet (Henry, 2019; Zhang et al., 2023). Both Comet and Black Sex-Linked hens are active foragers in pasture systems (Alig et al., 2023). Although direct comparisons are limited, commercial brown egg layers and hybrids generally show strong foraging motivation when given pasture access, suggesting breed differences likely had minimal impact on nutrient intake (Alig et al., 2023). Previous research indicates that feed type, rather than breed, has the greatest influence on egg nutrient profiles, particularly fatty acid content (Franco et al., 2020; Romero et al., 2024). Egg (Drabik et al., 2021)composition changes may result from both seasonal cycles and hen age, though cyclic variation is primarily observed in egg physical characteristics, with little evidence supporting breed influence on nutrient content (Christians, 2002).

Since this study spanned only one year, we cannot confirm whether these variations recur annually or are age-related. Future multi-year studies are needed to distinguish seasonal patterns from aging effects. Nevertheless, these challenges also highlight the adaptability of pasture-raising systems, where hens modify their reliance on forage and feed in response to environmental and seasonal changes. This emphasizes the importance of characterizing nutrient shifts across the season to optimize egg quality in free-living systems.

Future research should focus on adapting pasture-based systems to different regions and climates to assess the broader applicability of these findings. As this study was conducted on a single farm in Southern Ohio, the results may not be generalizable to all pasture-raised systems. Regional variations in climate, forage composition, and management practices could influence egg nutrient profiles, necessitating multi-location studies to better understand these effects. Practical strategies to address the challenges of hen intake control, predation, and nutrient consistency will be critical for optimizing production. One example of such a strategy is targeted feed supplementation during periods of low forage quality. In this study, fall feed supplementation

improved yolk omega-3 and vitamin levels, demonstrating its potential as a buffer against seasonal nutrient variability. Regionally adapted approaches like this may help producers maintain consistent egg quality year-round. Additionally, the egg industry can benefit from characterizing seasonal nutrient shifts to improve year-round egg quality, offering consumers reliable access to nutrient-dense eggs.

## 2.6 Conclusions

Seasonal environmental variations significantly influenced forage and egg nutrient profiles. Yolk n-3 fatty acids and vitamin A peaked in fall due to the forage-to-feed shift. Yolk antioxidant accumulation reflected forage quality and environmental conditions, while other nutrients like phenolics remained stable. Rotational grazing enriched egg composition by broadening the range of available nutrients, demonstrating the adaptability of pasture-raised hens to seasonal stressors and the importance of managing forage and feed intake for consistent, nutrient-dense egg production.

Future directions should aim on evaluating these findings across different regions to determine how broadly applicable they are across the country. In this study, the best months to purchase pasture-raised eggs were in the fall, as nutrient profiles were at their peak due to seasonal shifts. However, the significant variation observed in nutrient profiles highlights the need for greater consistency in pasture-raised egg production. While this study underscores the importance of evaluating seasonal shifts, it also points to the necessity of refining management practices to ensure nutrient-dense eggs year-round, offering consumers more reliable options for improved dietary benefits.

# CHAPTER III: CONCLUSIONS AND FUTURE DIRECTIONS

## 3.1 Conclusions

This thesis investigated how seasonal variation in pasture-based egg production systems influences the nutritional composition of egg yolks. By conducting monthly sampling of forage, soil, and eggs over a full grazing season in Southern Ohio, this work provides new insight into how environmental factors affect nutrient deposition, particularly omega-3 fatty acids, carotenoids, and fat-soluble vitamins, in regenerative poultry systems.

Chapter I presented a comprehensive review of the scientific literature on pasture-raised egg nutrition. It explored the roles of hen diet, forage access, and environmental conditions in shaping yolk composition and highlighted consistent findings that pasture-raised eggs contain higher levels of bioavailable nutrients than conventionally produced eggs. Importantly, this chapter also identified a key gap in the literature: the lack of research examining how these nutrient advantages fluctuate across the grazing season due to changes in pasture quality, plant diversity, and climate conditions.

Chapter II addressed this gap through a field-based study that evaluated how seasonal changes in forage composition and quality impact egg nutrient density. The results revealed clear temporal patterns. Vitamin E concentrations steadily increased over the season, while vitamin A peaked in late summer. Carotenoid levels were elevated in both midsummer and late autumn, and omega-3 fatty acid content—especially alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA)—was significantly higher in eggs collected during the fall. These seasonal trends aligned with improvements in pasture quality, particularly total digestible nutrients (TDN), and were confirmed through multivariate analyses that identified September to November as the period of highest egg nutrient density.

### 3.2 Future Directions

Overall, this thesis underscores the importance of considering seasonal variability when measuring and marketing the nutritional value of pasture-raised eggs. While these systems offer well-documented advantages regarding animal welfare**,** environmental sustainability**,** and enhanced omega-3 and antioxidant content, their nutritional outputs are not static. The nutrient composition of eggs—particularly concerning carotenoids, vitamin E, and long-chain omega-3 fatty acids—fluctuates throughout the grazing season, influenced by forage quality, environmental conditions, and pasture management practices (Chatzidimitriou, 2020; Daley et al., 2010; Krusinski, Maciel, et al., 2022)

Recognizing and accounting for these dynamics is essential to ensure consistent product quality**,** optimizing on-farm decision-making, and supporting transparent consumer labeling (Harmon et al., 2019; Jacob et al., 2018). Moreover, it enables producers to refine their pasture management and supplemental feeding strategies to better align with nutrient goals across different seasons.

Future research should investigate adaptive management approaches, including rotational grazing that promotes high-nutrient forages throughout the year (Lagrange, 2020). This includes exploring how hen foraging behavior impacts nutrient uptake and how seasonally driven changes in forage species composition translate to variations in egg quality. Additionally, more investigation is warranted to determine how observed differences in bioavailable nutrients in pasture-raised eggs impact long-term human health outcomes when incorporated into a habitual diet (Fleming et al., 2024).

By bridging the gap between ecological variation and food quality, this thesis contributes to the growing body of research that positions pasture-based poultry as a model for sustainable and

resilient food systems. These systems not only promote soil health and biodiversity but also produce eggs with superior nutritional profiles, supporting the growing demand for nutrient-dense, ethically produced, and locally sourced food**.**

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## APPENDIX A: SUPPLEMENTAL INFORMATION AND DATA

**Table A1. Proximate analysis of the forage samples by month and the layer hen feed[1]**

| Parameter | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|
| % Moisture | 86.20 ± 1.92 a | 76.00 ± 3.41 b | 75.53 ± 2.41 b | 66.93 ± 2.72 c | 78.30 ± 2.46 b | 78.90 ± 1.57 b | 77.47 ± 3.56 b | 72.57 ± 0.93 bc | <0.001 | 11.68 ± 0.5 |
| % Dry matter (DM) | 13.80 ± 1.92 c | 24.00 ± 3.41 b | 24.47 ± 2.41 b | 33.07 ± 2.72 a | 21.70 ± 2.46 b | 21.10 ± 1.57 b | 22.53 ± 3.56 b | 27.43 ± 0.93 ab | <0.001 | 88.35 ± 0.54 |
| Crude protein (% DM) | 16.37± 1.55 ab | 12.80 ± 0.62 ab | 17.30 ± 2.20 a | 12.10 ± 0.87 b | 14.10 ± 1.00 ab | 17.40 ± 1.76 a | 14.90 ± 2.29 ab | 12.47 ± 2.04 b | 0.003 | 18.02 ± 1.67 |
| ADF (% DM) | 36.07 ± 1.19 c | 39.67 ± 1.64 bc | 40.73 ± 2.84 abc | 45.97 ± 0.64 a | 43.80 ± 4.03 ab | 29.80 ± 1.61 d | 45.60 ± 0.79 a | 41.63 ± 1.07 abc | <0.001 | 6.65 ± 0.38 |
| NDF (% DM) | 58.70 ± 1.73 b | 62.97 ± 3.30 ab | 61.50 ± 2.43 ab | 68.10 ± 3.75 a | 62.27 ± 3.96 ab | 49.87 ± 3.32 c | 63.20 ± 3.85 ab | 66.2 ± 0.56 ab | <0.001 | 13.28 ± 1.15 |
| Lignin (% DM) | 4.87 ± 0.32 d | 6.27 ± 1.25 bcd | 6.40 ± 0.92 bcd | 10.03 ± 1.89 ab | 9.40 ± 2.13 abc | 5.57 ± 1.53 cd | 9.40 ± 2.13 a | 10.00 ± 1.22 ab | <0.001 | 2.32 ± 0.44 |
| Starch (% DM) | 0.37 ± 0.29 | 1.10 ± 0.26 | 1.30 ± 0.56 | 0.20 ± 0.00 | 0.50 ± 0.52 | 2.57 ± 2.76 | 1.30 ± 0.40 | 0.47 ± 0.25 | 0.183 | 38.82 ± 4.56 |
| Crude fat (% DM) | 3.27 ± 0.29 | 2.83 ± 0.21 | 3.37 ± 0.55 | 2.47 ± 0.47 | 3.33 ± 0.21 | 3.40 ± 0.53 | 2.43 ± 0.15 | 2.80 ± 0.46 | 0.024 | 4.50 ± 0.37 |
| Ash (% DM) | 11.26 ± 1.94 ab | 15.14 ± 4.59 a | 11.24 ± 1.16 ab | 9.15 ± 1.66 b | 9.35 ± 0.73 b | 10.04 ± 0.26 ab | 8.77 ± 1.30 b | 7.76 ± 0.38 b | 0.011 | 12.76 ± 1.36 |
| TDN (% DM) | 57.00 ± 2.00 ab | 47.33 ± 5.13 bc | 54.33 ± 2.08 abc | 47.00 ± 3.46 c | 51.33 ± 3.2 1 bc | 61.00 ± 2.65 a | 48.67 ± 2.89 bc | 49.33 ± 3.21 bc | 0.018 | 75.83 ± 1.72 |
| Metabolizable energy (mcal/kg) | 2.19 ± 0.08 ab | 1.74 ± 0.21 cd | 2.09 ± 0.12 abc | 1.67 ± 0.13 d | 1.91 ± 0.15 bcd | 2.35 ± 0.12 a | 1.78 ± 0.16 bcd | 1.79 ± 0.16 bcd | <0.001 | 3.02 ± 0.07 |
| Calcium (% DM) | 0.42 ± 0.09 bc | 0.33 ± 0.02 c | 0.69 ± 0.14 a | 0.54 ± 0.08 abc | 0.71 ± 0.13 a | 0.65 ± 0.02 ab | 0.72 ± 0.10 a | 0.57 ± 0.06 abc | 0.001 | 3.12 ± 0.76 |
| Phosphorus (% DM) | 0.29 ± 0.01 a | 0.19 ± 0.01 b | 0.30 ± 0.04 a | 0.17 ± 0.01 b | 0.24 ± 0.03 ab | 0.30 ± 0.02 a | 0.23 ± 0.06 ab | 0.22 ± 0.01 ab | <0.001 | 0.74 ± 0.11 |
| Magnesium (% DM) | 0.18 ± 0.03 b | 0.16 ± 0.01 b | 0.27 ± 0.02 b | 0.22 ± 0.04 b | 0.27 ± 0.03 b | 0.40 ± 0.09 a | 0.26 ± 0.05 b | 0.21 ± 0.02 b | <0.001 | 0.25 ± 0.03 |

[1]Means ± standard deviation ($n$ = 3 forage per month, $n$ = 6 layer hen feed samples) [2]Results of one-way ANOVA to compare forage by date. a-e, Means within a row for forage samples with different letters significantly differ ($p < 0.05$). DM, dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber; TDN, total digestible nutrients

**Table A1. (cont'd)**

| Parameter | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|
| Potassium (% DM) | 2.91 ± 0.42 a | 1.10 ± 0.17 b | 2.90 ± 0.49 a | 1.09 ± 0.08 b | 1.61 ± 0.34 b | 2.83 ± 0.09 a | 1.11 ± 0.66 b | 0.83 ± 0.23 b | <0.001 | 0.75 ± 0.13 |
| Sodium (% DM) | 0.01 ± 0.00 bc | 0.04 ± 0.00 a | 0.02 ± 0.01 b | 0.02 ± 0.01 bc | 0.01 ± 0.00 c | 0.02 ± 0.00 bc | 0.01 ± 0.01 bc | 0.01 ± 0.00 bc | <0.001 | 0.24 ± 0.06 |
| Sulfur (% DM) | 0.30 ± 0.04 a | 0.15 ± 0.07 b | 0.28 ± 0.05 a | 0.12 ± 0.02 b | 0.2 ± 0.06 ab | 0.31 ± 0.04 a | 0.14 ± 0.03 b | 0.23 ± 0.01 ab | <0.001 | 0.28 ± 0.05 |
| Chloride (% DM) | 0.78 ± 0.21 b | 0.68 ± 0.21 b | 0.88 ± 0.36 b | 0.65 ± 0.09 ab | 0.89 ± 0.11 b | 1.23 ± 0.13 a | 0.59 ± 0.24 ab | 0.52 ± 0.13 b | 0.015 | 0.44 ± 0.03 |
| Iron (ppm) | 109.00 ± 56.00 b | 901.00 ± 466.00 a | 160.00 ± 51.00 b | 526.00 ± 118.00 ab | 196.00 ± 140.50 b | 49.00 ± 22.00 b | 409.00 ± 134.50 ab | 309.00 ± 126.00 ab | 0.010 | 353.67 ± 104.25 |
| Zinc (ppm) | 3.00 ± 0.50 | 8.00 ± 1.50 | 8.00 ± 2.00 | 7.00 ± 1.50 | 7.00 ± 0.50 | 6.00 ± 1.50 | 7.00 ± 1.00 | 7.00 ± 0.50 | 0.170 | 115.00 ± 19.26 |
| Copper (ppm) | 1.00 ± 0.00 | 1.50 ± 0.50 | 2.00 ± 0.00 | 2.00 ± 0.50 | 2.00 ± 0.00 | 2.00 ± 0.50 | 2.00 ± 0.50 | 2.00 ± 0.50 | 0.061 | 21.83 ± 4.36 |
| Manganese (ppm) | 10.00 ± 3.50 c | 95.00 ± 21.50 a | 42.00 ± 14.00 abc | 60.00 ± 12.50 ab | 31.00 ± 11.50 bc | 24.00 ± 8.50 c | 47.00 ± 17.00 abc | 73.00 ± 8.50 ab | 0.012 | 106.50 ± 25.25 |
| Molybdenum (ppm) | NA ± NA | 0.50 ± 0.15 | 0.40 ± 0.15 | 0.40 ± 0.25 | NA ± NA | NA ± NA | 0.40 ± 0.10 | 0.30 ± 0.05 | 0.262 | 2.15 ± 1.11 |

**Table A2. Fatty acid analysis of the forage samples by month and the layer hen feed (g per 100g)[1]**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Caprylic | 8:0 | 0.025 ± 0.004 a | 0.041 ± 0.002 a | 0.071 ± 0.011 a | 0.07 ± 0.008 a | 0.057 ± 0.014 a | 0.056 ± 0.011 a | 0.057 ± 0.007 a | 0.062 ± 0.122 a | 0.024 | 0.168 ± 0.070 |
| Capric | 10:0 | 0.005 ± 0.001 | 0.008 ± 0.009 | 0.007 ± 0.002 | 0.007 ± 0.004 | 0.005 ± 0.001 | 0.005 ± 0.001 | 0.005 ± 0.001 | 0.034 ± 0.073 | 0.131 | 0.014 ± 0.004 |
| Undecanoic | 11:0 | 0.001 ± 0.000 | 0.001 ± 0.000 | 0.001 ± 0.001 | 0.001 ± 0.000 | 0.001 ± 0.000 | 0.001 ± 0.000 | 0.001 ± 0.000 | 0.001 ± 0.003 | 0.818 | 0.002 ± 0.001 |
| Lauric | 12:0 | 0.013 ± 0.003 a | 0.047 ± 0.003 a | 0.046 ± 0.023 a | 0.035 ± 0.004 a | 0.038 ± 0.004 a | 0.037 ± 0.005 a | 0.023 ± 0.005 a | 0.035 ± 0.051 a | 0.037 | 0.025 ± 0.010 |
| Tridecanoic | 13:0 | LOD | 0.001 ± 0.000 | LOD | LOD | LOD | LOD | LOD | LOD | 0.362 | 0.002 ± 0.001 |
| Myristic | 14:0 | 0.038 ± 0.012 | 0.065 ± 0.027 | 0.054 ± 0.03 | 0.064 ± 0.007 | 0.033 ± 0.001 | 0.052 ± 0.006 | 0.067 ± 0.024 | 0.059 ± 0.064 | 0.056 | 1.104 ± 0.435 |
| Myristoleic | 14:1 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Pentadecanoic | 15:0 | 0.015 ± 0.007 b | 0.022 ± 0.003 ab | 0.015 ± 0.002 b | 0.042 ± 0.005 a | 0.007 ± 0.001 b | 0.008 ± 0.001 b | 0.017 ± 0.01 a b | 0.006 ± 0.015 b | 0.034 | 0.086 ± 0.033 |
| Palmitic | 16:0 | 1.136 ± 0.500 | 1.533 ± 0.169 | 2.612 ± 0.344 | 2.554 ± 0.213 | 1.425 ± 0.263 | 1.722 ± 0.205 | 1.223 ± 0.562 | 0.643 ± 2.714 | 0.113 | 21.411 ± 9.568 |
| Palmiteladic | 16:1 n-9t | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Palmitoleic | 16:1 n-7 | 0.103 ± 0.038 | 0.050 ± 0.005 | 0.117 ± 0.021 | 0.055 ± 0.006 | 0.041 ± 0.016 | 0.062 ± 0.022 | 0.016 ± 0.009 | 0.011 ± 0.125 | 0.060 | 0.082 ± 0.038 |
| | 16:1 n-9 | 0.018 ± 0.009 c | 0.059 ± 0.011 abc | 0.048 ± 0.003 abc | 0.127 ± 0.014 a | 0.029 ± 0.003 bc | 0.027 ± 0.029 abc | 0.115 ± 0.027 abc | 0.085 ± 0.078 ab | 0.012 | 1.635 ± 0.779 |
| Heptadecanoic | 17:0 | 0.015 ± 0.009 | 0.028 ± 0.003 | 0.045 ± 0.007 | 0.057 ± 0.004 | 0.024 ± 0.001 | 0.023 ± 0.003 | 0.028 ± 0.011 | 0.015 ± 0.03 | 0.123 | 0.177 ± 0.077 |
| c10-heptadecanoic | 17:1 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Stearic | 18:0 | 0.166 ± 0.078 a | 0.222 ± 0.030 a | 0.459 ± 0.055 a | 0.529 ± 0.054 a | 0.296 ± 0.027 a | 0.349 ± 0.029 a | 0.399 ± 0.097 a | 0.223 ± 0.539 a | 0.049 | 3.461 ± 1.365 |
| Eladic | 18:1 n-9t | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |

[1]Means ± standard deviation n = 3 forage replicates per month and layer hen feed n=6 [2]Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ $p < 0.05$. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; OCFA, odd-chain fatty acids; FA, fatty acids.

**Table A2. (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oleic | 18:1 n-9 | 0.203 ± 0.080 a | 0.351 ± 0.041 a | 0.560 ± 0.051 a | 0.872 ± 0.109 a | 0.393 ± 0.032 a | 0.461 ± 0.114 a | 0.496 ± 0.18 a | 0.643 ± 0.768 a | 0.007 | 30.802 ± 16.904 |
| | 18:1 n-11 | 0.254 ± 0.190 b | 0.467 ± 0.086 b | 0.227 ± 0.214 b | 1.247 ± 0.157 a | 0.171 ± 0.026 b | 0.057 ± 0.076 b | 0.410 ± 0.162 b | 0.248 ± 0.228 b | 0.037 | 9.440 ± 4.874 |
| Linoleic | 18:2 n-6 | 1.528 ± 0.579 | 1.564 ± 0.064 | 2.219 ± 0.31 | 2.917 ± 0.100 | 1.282 ± 0.316 | 1.695 ± 0.317 | 1.089 ± 0.16 | 0.799 ± 2.056 | 0.082 | 84.986 ± 41.376 |
| ALA | 18:3 n-3 | 4.256 ± 1.613 | 3.744 ± 0.252 | 6.681 ± 1.20 | 3.987 ± 0.561 | 3.86 ± 1.957 | 4.899 ± 1.670 | 0.940 ± 0.387 | 0.761 ± 8.744 | 0.117 | 5.530 ± 2.200 |
| GLA | 18:3 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Arachidic | 20:0 | 0.107 ± 0.063 | 0.115 ± 0.014 | 0.146 ± 0.047 | 0.141 ± 0.014 | 0.074 ± 0.01 | 0.075 ± 0.015 | 0.082 ± 0.021 | 0.062 ± 0.196 | 0.122 | 0.769 ± 0.471 |
| Eicosenoic | 20:1 n-9 | 0.022 ± 0.006 | 0.015 ± 0.002 | 0.026 ± 0.006 | 0.033 ± 0.003 | 0.023 ± 0.002 | 0.022 ± 0.003 | 0.018 ± 0.004 | 0.017 ± 0.037 | 0.080 | 0.833 ± 0.512 |
| Eicosedienoic | 20:2 n-6 | 0.010 ± 0.002 ab | 0.007 ± 0.001 b | 0.014 ± 0.006 ab | 0.018 ± 0.003 a | 0.006 ± 0.002 b | 0.008 ± 0.004 ab | 0.004 ± 0.001 b | 0.005 ± 0.002 b | 0.041 | 0.136 ± 0.110 |
| Eicosatrenoic | 20:3 n-3 | 0.018 ± 0.006 | 0.013 ± 0.001 | 0.03 ± 0.002 | 0.021 ± 0.005 | 0.020 ± 0.005 | 0.023 ± 0.002 | 0.023 ± 0.004 | 0.021 ± 0.037 | 0.075 | 0.070 ± 0.028 |
| DGLA | 20:3 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Mead | 20:9 n-9 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Arachidonic | 20:4 n-6 | LOD | 0.015 ± 0.004 | 0.035 ± 0.006 | 0.032 ± 0.005 | 0.023 ± 0.008 | 0.022 ± 0.003 | 0.028 ± 0.009 | 0.026 ± 0.044 | 0.078 | 0.248 ± 0.108 |
| EPA | 20:5 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | 1.495 ± 0.903 |
| Behenic | 22:0 | 0.144 ± 0.066 | 0.142 ± 0.014 | 0.273 ± 0.017 | 0.260 ± 0.028 | 0.175 ± 0.039 | 0.162 ± 0.024 | 0.189 ± 0.024 | 0.157 ± 0.343 | 0.124 | 0.738 ± 0.299 |
| DTA | 22:4 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| DPA | 22:5 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | 2.612 ± 1.952 |
| DPA | 22:5 n-6 | 0.068 ± 0.003 b | 0.056 ± 0.019 b | 0.098 ± 0.005 ab | 0.132 ± 0.030 a | 0.080 ± 0.003 ab | 0.082 ± 0.010 ab | LOD | LOD | 0.035 | 0.377 ± 0.165 |
| DHA | 22:6 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | 1.513 ± 0.856 |
| Lignoceric | 24:0 | 0.147 ± 0.061 a | 0.131 ± 0.013 a | 0.203 ± 0.089 a | 0.196 ± 0.025 a | 0.063 ± 0.019 a | 0.057 ± 0.011 a | 0.086 ± 0.016 a | 0.102 ± 0.179 a | 0.034 | 0.489 ± 0.361 |

**Table A2. (cont'd)**

| Fatty Acid Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|
| Total SFA | 1.798 ± 0.795 | 2.307 ± 0.204 | 3.908 ± 0.582 | 3.95 ± 0.311 | 2.348 ± 0.227 | 2.647 ± 0.235 | 2.292 ± 0.709 | 1.308 ± 4.284 | 0.131 | 28.111 ± 12.833 |
| Total MUFA | 0.600 ± 0.322 | 0.993 ± 0.109 | 0.975 ± 0.178 | 2.142 ± 0.149 | 0.687 ± 0.050 | 0.625 ± 0.198 | 1.012 ± 0.348 | 1.000 ± 1.232 | 0.088 | 40.898 ± 26.029 |
| Total PUFA | 5.875 ± 2.235 | 5.374 ± 0.225 | 9.087 ± 1.503 | 6.975 ± 0.703 | 5.212 ± 2.271 | 6.724 ± 1.966 | 2.252 ± 0.474 | 1.55 ± 10.854 | 0.155 | 95.122 ± 50.191 |
| Total n-6 | 1.601 ± 0.616 | 1.648 ± 0.084 | 2.376 ± 0.304 | 2.967 ± 0.139 | 1.324 ± 0.314 | 1.802 ± 0.296 | 1.122 ± 0.167 | 0.823 ± 2.102 | 0.084 | 81.700 ± 48.876 |
| Total n-3 | 4.275 ± 1.619 | 3.757 ± 0.253 | 6.711 ± 1.198 | 4.008 ± 0.565 | 3.888 ± 1.958 | 4.922 ± 1.669 | 0.970 ± 0.387 | 0.782 ± 8.78 | 0.117 | 13.422 ± 5.305 |
| n-6:n-3 ratio | 0.341 ± 0.033 | 0.420 ± 0.041 | 0.341 ± 0.02 | 0.740 ± 0.077 | 0.341 ± 0.055 | 0.348 ± 0.043 | 1.322 ± 0.345 | 0.981 ± 0.652 | 0.051 | 6.805 ± 3.394 |
| Total OCFA | 0.031 ± 0.015 | 0.051 ± 0.005 | 0.06 ± 0.009 | 0.099 ± 0.010 | 0.032 ± 0.002 | 0.031 ± 0.003 | 0.046 ± 0.019 | 0.021 ± 0.048 | 0.096 | 0.259 ± 0.110 |
| Total FA | 8.273 ± 3.352 | 8.674 ± 0.537 | 14.801 ± 1.847 | 13.328 ± 0.996 | 8.278 ± 2.533 | 9.839 ± 2.32 | 6.237 ± 1.175 | 3.652 ± 16.268 | 0.121 | 164.131 ± 88.932 |

**Table A3. Fatty acid analysis of the forage samples by month and the layer hen feed (percent of total fatty acids)[1]**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Caprylic | 8:0 | 0.289 ± 0.061 c | 0.467 ± 0.006 bc | 0.481 ± 0.159 bc | 0.551 ± 0.05 b c | 0.763 ± 0.275 abc | 0.58 ± 0.186 b c | 1.057 ± 0.176 ab | 1.262 ± 0.496 a | 0.018 | 0.175 ± 0.048 |
| Capric | 10:0 | 0.051 ± 0.025 b | 0.084 ± 0.112 ab | 0.058 ± 0.012 b | 0.047 ± 0.027 b | 0.066 ± 0.022 b | 0.051 ± 0.02 b | 0.097 ± 0.018 b | 0.425 ± 0.401 a | 0.038 | 0.010 ± 0.004 |
| Undecanoic | 11:0 | 0.008 ± 0.001 b | 0.013 ± 0.002 ab | 0.010 ± 0.005 ab | 0.007 ± 0.003 b | 0.009 ± 0.002 b | 0.006 ± 0.002 b | 0.014 ± 0.003 ab | 0.017 ± 0.003 a | 0.028 | 0.002 ± 0.001 |
| Lauric | 12:0 | 0.131 ± 0.025 b | 0.506 ± 0.048 ab | 0.298 ± 0.127 ab | 0.249 ± 0.042 ab | 0.453 ± 0.142 ab | 0.387 ± 0.114 ab | 0.506 ± 0.088 ab | 0.575 ± 0.349 a | 0.040 | 0.016 ± 0.004 |
| Tridecanoic | 13:0 | LOD | 0.006 ± 0.004 a | LOD | 0.001 ± 0.000 b | LOD | LOD | LOD | LOD | 0.027 | 0.002 ± 0.000 |
| Myristic | 14:0 | 0.327 ± 0.084 b | 0.697 ± 0.347 ab | 0.347 ± 0.173 b | 0.524 ± 0.064 ab | 0.399 ± 0.097 b | 0.436 ± 0.073 b | 1.630 ± 0.399 a | 1.000 ± 0.629 ab | 0.026 | 0.722 ± 0.217 |
| Myristoleic | 14:1 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Pentadecanoic | 15:0 | 0.152 ± 0.030 bc | 0.249 ± 0.013 ab | 0.114 ± 0.017 c | 0.300 ± 0.033 a | 0.085 ± 0.041 c | 0.076 ± 0.018 c | 0.351 ± 0.116 a | 0.136 ± 0.032 bc | 0.005 | 0.057 ± 0.021 |
| Palmitic | 16:0 | 14.045 ± 0.363 b | 16.756 ± 1.411 ab | 18.061 ± 1.279 ab | 20.129 ± 0.571 ab | 17.212 ± 1.486 ab | 16.759 ± 1.653 ab | 19.604 ± 5.46 a | 16.591 ± 0.936 ab | 0.017 | 13.049 ± 0.879 |
| Palmiteladic | 16:1 n-9t | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Palmitoleic | 16:1 n-7 | 1.158 ± 0.05 a | 0.577 ± 0.085 bc | 0.764 ± 0.073 b | 0.448 ± 0.031 bc | 0.507 ± 0.028 bc | 0.628 ± 0.068 bc | 0.260 ± 0.096 c | 0.320 ± 0.257 bc | 0.014 | 0.050 ± 0.005 |
| | 16:1 n-9 | 0.220 ± 0.014 c | 0.680 ± 0.091 bc | 0.327 ± 0.064 c | 1.043 ± 0.131 bc | 0.388 ± 0.101 c | 0.188 ± 0.294 c | 1.954 ± 0.183 a | 1.616 ± 0.865 ab | 0.010 | 1.006 ± 0.189 |
| Heptadecanoic | 17:0 | 0.189 ± 0.024 c | 0.319 ± 0.023 bc | 0.286 ± 0.021 bc | 0.408 ± 0.021 ab | 0.288 ± 0.054 bc | 0.237 ± 0.058 c | 0.483 ± 0.094 a | 0.374 ± 0.105 bc | 0.013 | 0.109 ± 0.012 |
| c10-heptadecanoic | 17:1 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Stearic | 18:0 | 2.003 ± 0.101 c | 2.563 ± 0.184 c | 3.357 ± 0.262 c | 3.728 ± 0.251 bc | 3.922 ± 0.906 bc | 3.039 ± 0.575 c | 6.864 ± 0.496 a | 6.103 ± 1.269 ab | 0.010 | 2.180 ± 0.342 |
| Eladic | 18:1 n-9t | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |

[1]Means ± standard deviation n = 3 forage replicates per month and layer hen feed n=6 [2]Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ $p < 0.05$. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; OCFA, odd-chain fatty acids; FA, fatty acids.

**Table A3. (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oleic | 18:1 n-9 | 2.497 ± 0.074 c | 4.16 ± 0.261 bc | 3.578 ± 0.811 bc | 6.373 ± 0.458 abc | 5.285 ± 1.163 bc | 4.594 ± 0.196 bc | 12.134 ± 2.992 ab | 14.636 ± 5.969 a | 0.006 | 17.793 ± 1.634 |
| | 18:1 n-11 | 3.073 ± 0.850 b | 5.037 ± 0.870 ab | 1.452 ± 1.310 b | 9.776 ± 1.628 a | 1.798 ± 0.283 b | 0.589 ± 0.464 b | 10.030 ± 2.703 a | 2.882 ± 2.620 b | 0.007 | 5.572 ± 0.577 |
| Linoleic | 18:2 n-6 | 17.021 ± 0.827 ab | 18.511 ± 1.065 ab | 15.243 ± 0.304 b | 21.884 ± 0.885 a | 15.49 ± 0.642 b | 15.935 ± 1.057 ab | 19.842 ± 2.756 ab | 21.613 ± 4.19 ab | 0.044 | 50.570 ± 1.517 |
| ALA | 18:3 n-3 | 51.447 ± 1.524 a | 44.399 ± 1.974 abc | 48.147 ± 3.484 abc | 29.914 ± 2.009 bcd | 46.635 ± 6.275 ab | 50.547 ± 3.937 a | 15.078 ± 3.279 d | 22.263 ± 18.106 cd | 0.018 | 3.540 ± 0.780 |
| GLA | 18:3 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Arachidic | 20:0 | 1.382 ± 0.164 a | 1.348 ± 0.144 ab | 0.930 ± 0.243 ab | 1.123 ± 0.053 ab | 0.989 ± 0.256 ab | 0.642 ± 0.211 b | 1.609 ± 0.224 a | 1.166 ± 0.455 ab | 0.031 | 0.424 ± 0.088 |
| Eicosenoic | 20:1 n-9 | 0.267 ± 0.023 ab | 0.184 ± 0.016 b | 0.166 ± 0.065 b | 0.235 ± 0.011 ab | 0.300 ± 0.071 ab | 0.193 ± 0.028 b | 0.378 ± 0.056 ab | 0.475 ± 0.128 a | 0.038 | 0.466 ± 0.085 |
| Eicosedienoic | 20:2 n-6 | 0.101 ± 0.017 | 0.076 ± 0.008 | 0.117 ± 0.046 | 0.132 ± 0.015 | 0.077 ± 0.042 | 0.086 ± 0.020 | 0.069 ± 0.054 | 0.023 ± 0.044 | 0.260 | 0.071 ± 0.037 |
| Eicosatrenoic | 20:3 n-3 | 0.221 ± 0.021 ab | 0.160 ± 0.009 b | 0.203 ± 0.042 ab | 0.159 ± 0.02 b | 0.252 ± 0.09 a b | 0.234 ± 0.051 ab | 0.483 ± 0.109 a | 0.443 ± 0.186 a | 0.028 | 0.058 ± 0.016 |
| DGLA | 20:3 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Mead | 20:9 n-9 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |
| Arachidonic | 20:4 n-6 | LOD | 0.167 ± 0.026 b | 0.238 ± 0.09 a b | 0.24 ± 0.021 a b | 0.312 ± 0.136 ab | 0.207 ± 0.126 b | 0.587 ± 0.113 a | 0.675 ± 0.222 a | 0.016 | 0.155 ± 0.023 |
| EPA | 20:5 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | 0.859 ± 0.199 |
| Behenic | 22:0 | 1.872 ± 0.213 bc | 1.729 ± 0.102 c | 1.843 ± 0.392 bc | 2.052 ± 0.105 abc | 2.355 ± 0.769 abc | 1.668 ± 0.484 c | 3.352 ± 0.51 a b | 3.982 ± 1.14 a | 0.046 | 0.464 ± 0.079 |
| DTA | 22:4 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | LOD |

**Table A3 (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DPA | 22:5 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | 1.374 ± 0.547 |
| | 22:5 n-6 | 0.501 ± 0.384 | 0.606 ± 0.207 | 0.667 ± 0.06 | 0.832 ± 0.57 | 0.665 ± 0.525 | 0.848 ± 0.226 | LOD | LOD | 0.149 | 0.236 ± 0.040 |
| DHA | 22:6 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | NA | 0.893 ± 0.230 |
| Lignoceric | 24:0 | 1.678 ± 0.109 ab | 1.592 ± 0.087 ab | 1.300 ± 0.499 ab | 1.496 ± 0.192 ab | 0.850 ± 0.313 ab | 0.530 ± 0.166 b | 1.612 ± 0.163 ab | 1.393 ± 0.833 a | 0.043 | 0.255 ± 0.101 |
| Total SFA | | 22.036 ± 0.563 c | 26.594 ± 0.73 bc | 27.497 ± 2.555 bc | 30.767 ± 0.594 abc | 28.087 ± 4.015 bc | 24.414 ± 3.558 bc | 36.756 ± 4.371 a | 34.694 ± 4.34 ab | 0.013 | 17.375 ± 1.392 |
| Total MUFA | | 7.257 ± 0.785 bc | 10.987 ± 0.839 bc | 7.584 ± 1.149 bc | 17.569 ± 1.591 abc | 8.290 ± 1.586 bc | 6.350 ± 0.372 c | 24.772 ± 5.831 a | 19.965 ± 9.31 ab | 0.009 | 24.888 ± 1.728 |
| Total PUFA | | 71.015 ± 1.194 a | 62.325 ± 1.523 abc | 64.919 ± 3.704 abc | 52.333 ± 1.331 bcd | 63.623 ± 5.601 ab | 69.374 ± 3.255 a | 38.84 ± 1.643 d | 45.34 ± 13.65 cd | 0.013 | 57.738 ± 0.662 |
| Total n-6 | | 17.614 ± 1.218 | 19.209 ± 1.181 | 16.494 ± 0.224 | 22.430 ± 0.784 | 15.997 ± 0.954 | 17.233 ± 1.312 | 20.561 ± 2.886 | 22.455 ± 4.359 | 0.071 | 51.032 ± 1.502 |
| Total n-3 | | 51.667 ± 1.546 a | 44.566 ± 1.979 abc | 48.425 ± 3.48 abc | 30.073 ± 2.03 bcd | 46.971 ± 6.227 ab | 50.781 ± 3.886 a | 15.637 ± 3.209 d | 22.885 ± 18.009 cd | 0.018 | 6.705 ± 1.055 |
| n-6:n-3 ratio | | 0.341 ± 0.033 | 0.420 ± 0.041 | 0.341 ± 0.020 | 0.740 ± 0.077 | 0.341 ± 0.055 | 0.348 ± 0.043 | 1.322 ± 0.345 | 0.981 ± 0.652 | 0.051 | 7.791 ± 1.390 |
| Total OCFA | | 0.369 ± 0.038 c | 0.592 ± 0.027 bc | 0.416 ± 0.033 c | 0.718 ± 0.053 ab | 0.382 ± 0.097 c | 0.322 ± 0.077 c | 0.847 ± 0.206 a | 0.529 ± 0.134 bc | 0.008 | 0.168 ± 0.034 |

**Table A4. Antioxidant profile of the forage by month and the layer hen feed[1]**

| Parameter | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] | Layer Hen Feed |
|---|---|---|---|---|---|---|---|---|---|---|
| Vitamin A (ng/g DM) | ND | ND | ND | ND | ND | ND | ND | ND | NA | 10623.67 ± 767.76 |
| Beta-carotene (ug/g DM) | NA | NA | NA | NA | NA | NA | 3.16 ± 3.32 | 5.28 ± 5.47 | NA | NA |
| Vitamin E (ug/g DM) | 9.90 ± 1.48 c | 28.10 ± 0.97 bc | 108.72 ± 56.69 ab | 29.78 ± 3.86 bc | 53.22 ± 20.84 bc | 103.40 ± 51.02 ab | 104.17 ± 25.50 ab | 158.94 ± 13.34 a | <0.001 | 11.21 ± 10.76 |
| Chlorophyll a (ug/g DM) | 2748.64 ± 195.42 a | 1557.10 ± 270.13 abc | 2327.67 ± 228.46 ab | 1138.18 ± 166.5 bc | 2614.71 ± 1121.41 ab | 2278.5 ± 410.71 ab | 347.74 ± 165.31 c | 716.74 ± 778.80 c | <0.001 | 17.22 ± 5.30 |
| Chlorophyll b (ug/g DM) | 976.30 ± 71.32 a | 556.26 ± 117.71 abc | 781.41 ± 103.01 ab | 493.34 ± 5 9.05 bc | 927.66 ± 324.63 ab | 791.32 ± 149.99 ab | 149.22 ± 15.25 c | 269.51 ± 223.06 c | <0.001 | 22.10 ± 8.25 |
| Total Carotenoids (ug/g DM) | 765.92 ± 43.66 a | 461.03 ± 94.35 abcd | 626.32 ± 85.66 abc | 270.47 ± 36.25 bcd | 757.95 ± 378.49 ab | 671.46 ± 113.89 abc | 73.06 ± 5.80 d | 219.65 ± 251.93 cd | 0.001 | 14.47 ± 2.67 |
| Total phenolic content (mg/g DM) | 4.143 ± 0.474 a | 2.284 ± 0.606 bcd | 2.019 ± 0.526 bcd | 1.302 ± 0.272 cd | 2.571 ± 0.554 abc | 3.644 ± 0.735 ab | 0.546 ± 0.471 d | 1.606 ± 0.493 cd | 0.008 | 0.91 ± 0.25 |

[1]Means ± standard deviation ($n$ = 3 forage replicates per month, $n$ = 6 layer hen feed samples) [2]Results of one-way ANOVA to compare forage by date. a-e, Means within a row for forage samples with different letters significantly differ ($p < 0.05$). DM, dry matter; ND, not detected; NA, value not determined for specific collection

**Table A5. Antioxidant profile of the egg yolks by month[1]**

| Parameter | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|
| Vitamin A (ug/g FW) | 4.04 ± 0.63 d | 4.19 ± 0.38 d | 6.97 ± 0.67 bc | 9.03 ± 1.89 ab | 10.80 ± 3.88 a | 6.62 ± 1.97 c | 7.77 ± 0.63 bc | 2.85 ± 0.39 d | <0.001 |
| Vitamin E (ug/g FW) | 4.65 ± 6.01 e | 14.90 ± 19.23 de | 33.52 ± 18.06 d | 55.12 ± 9.79 c | 68.82 ± 13.02 bc | 81.42 ± 17.57 b | 118.06 ± 23.89 a | 25.72 ± 6.90 d | <0.001 |
| Total carotenoids (ug/g FW) | 16.34 ± 7.95 c | 20.64 ± 5.65 c | 34.95 ± 11.74 abc | 48.94 ± 12.19 a | 29.92 ± 12.03 bc | 44.39 ± 28.13 ab | 39.59 ± 6.57 ab | 49.69 ± 19.44 a | <0.001 |
| Beta carotene (ug/g FW) | 14.88 ± 7.34 c | 18.85 ± 5.20 c | 31.75 ± 11.03 abc | 44.85 ± 10.99 a | 27.37 ± 11.01 bc | 40.75 ± 25.93 ab | 36.25 ± 6.84 ab | 45.23 ± 17.72 a | <0.001 |
| Total phenolic content (mg GAE/g FW) | 0.14 ± 0.02 ab | 0.13 ± 0.02 ab | 0.11 ± 0.01 b | 0.14 ± 0.02 a | 0.14 ± 0.02 a | 0.14 ± 0.02 ab | 0.13 ± 0.03 ab | 0.14 ± 0.03 ab | 0.019 |

[1]Means ± standard deviation (n = 24 eggs pooled into n = 12 replicates per month) [2]Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ ($p < 0.05$). FW, fresh weight; GAE, gallic acid equivalents

**Table A6.** Egg yolk fatty acids and cholesterol content by month (g of fatty acid per 100 g of fresh egg yolk)[1]

| Fatty Acid | Carbon Number | May | Jun | Jul | Aupg | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| Caprylic | 8:0 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| Capric | 10:0 | 0.001 ± 0.001 c | 0.001 ± 0.001 bc | 0.002 ± 0.001 ab | 0.002 ± 0.001 a | 0.001 ± 0.001 c | 0.001 ± 0.001 c | 0.001 ± 0.001 c | 0.001 ± 0.001 c | <0.001 |
| Undecanoic | 11:0 | LOD | 0.001 ± 0.001 ab | 0.000 ± 0.001 abc | 0.001 ± 0.001 a | LOD | LOD | LOD | LOD | <0.001 |
| Lauric | 12:0 | LOD | 0.000 ± 0.001 b | 0.001 ± 0.001 a | 0.001 ± 0.001 a | 0.000 ± 0.001 bc | LOD | LOD | LOD | <0.001 |
| Tridecanoic | 13:0 | 0.002 ± 0.001 ab | 0.002 ± 0.001 ab | 0.002 ± 0.001 a | 0.002 ± 0.000 ab | 0.002 ± 0.000 ab | 0.001 ± 0.001 c | 0.002 ± 0.000 b | 0.001 ± 0.000 c | <0.001 |
| Myristic | 14:0 | 0.061 ± 0.014 ab | 0.065 ± 0.019 a | 0.05 ± 0.009 abc | 0.047 ± 0.009 bc | 0.057 ± 0.014 ab | 0.041 ± 0.013 c | 0.065 ± 0.008 a | 0.036 ± 0.007 c | <0.001 |
| Myristoleic | 14:1 | 0.010 ± 0.003 cd | 0.015 ± 0.004 ab | 0.008 ± 0.004 d | 0.011 ± 0.003 bcd | 0.014 ± 0.005 abc | 0.013 ± 0.005 abcd | 0.016 ± 0.003 a | 0.008 ± 0.002 d | <0.001 |
| Pentadecanoic | 15:0 | 0.014 ± 0.002 ab | 0.012 ± 0.004 abc | 0.011 ± 0.002 bc | 0.010 ± 0.002 c | 0.015 ± 0.003 a | 0.009 ± 0.003 c | 0.015 ± 0.003 a | 0.009 ± 0.002 c | <0.001 |
| Palmitic | 16:0 | 4.939 ± 1.097 a | 3.7 ± 0.478 bcd | 3.987 ± 0.388 bc | 3.405 ± 0.385 cd | 4.328 ± 0.756 ab | 2.956 ± 0.726 d | 4.12 ± 0.65 abc | 3.052 ± 0.507 d | <0.001 |
| Palmiteladic | 16:1 n-9t | 0.009 ± 0.002 bc | 0.007 ± 0.002 c | 0.007 ± 0.002 c | 0.007 ± 0.001 c | 0.011 ± 0.004 ab | 0.009 ± 0.002 bc | 0.013 ± 0.002 a | 0.007 ± 0.002 c | <0.001 |
| Palmitoleic | 16:1 n-7 | 0.146 ± 0.035 a | 0.078 ± 0.015 bcd | 0.093 ± 0.020 b | 0.059 ± 0.012 d | 0.1 ± 0.027 b | 0.064 ± 0.019 cd | 0.09 ± 0.018 bc | 0.074 ± 0.014 bcd | <0.001 |
| | 16:1 n-9 | 0.492 ± 0.094 abcd | 0.5 ± 0.097 abc | 0.362 ± 0.103 cd | 0.429 ± 0.069 bcd | 0.556 ± 0.159 ab | 0.465 ± 0.143 bcd | 0.626 ± 0.099 a | 0.36 ± 0.084 d | <0.001 |
| Heptadecanoic | 17:0 | 0.039 ± 0.011 ab | 0.030 ± 0.003 bc | 0.032 ± 0.007 bc | 0.024 ± 0.004 c | 0.046 ± 0.014 a | 0.028 ± 0.004 c | 0.042 ± 0.008 a | 0.030 ± 0.006 bc | <0.001 |
| c10-heptadecanoic | 17:1 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| Stearic | 18:0 | 0.039 ± 0.008 ab | 0.03 ± 0.009 bc | 0.032 ± 0.005 bc | 0.024 ± 0.003 c | 0.044 ± 0.01 a | 0.029 ± 0.007 c | 0.042 ± 0.006 a | 0.03 ± 0.005 bc | <0.001 |
| Eladic | 18:1 n-9t | 0.034 ± 0.008 abc | 0.029 ± 0.006 bcd | 0.026 ± 0.007 cd | 0.019 ± 0.004 d | 0.038 ± 0.017 ab | 0.036 ± 0.013 abc | 0.042 ± 0.009 a | 0.03 ± 0.005 abcd | <0.001 |

[1]Means ± standard deviation n = 24 eggs pooled into n = 12 replicates per month [2]Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ $p < 0.05$. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; OCFA, odd-chain fatty acids; FA, fatty acids.

**Table A6. (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| Oleic | 18:1 n-9 | 8.466 ± 1.589 a | 5.424 ± 0.565 bc | 6.398 ± 0.653 b | 5.657 ± 0.73 b | 6.235 ± 1.192 b | 4.217 ± 1.355 c | 6.077 ± 0.758 b | 4.331 ± 0.713 c | <0.001 |
| | 18:1 n-11 | 0.323 ± 0.060 ab | 0.235 ± 0.030 cd | 0.189 ± 0.04 cd | 0.18 ± 0.029 d | 0.374 ± 0.137 a | 0.27 ± 0.074 bc | 0.389 ± 0.058 a | 0.236 ± 0.048 cd | <0.001 |
| Linoleic | 18:2 n-6 | 3.405 ± 0.995 a | 2.016 ± 0.540 bc | 3.034 ± 1.176 a | 1.892 ± 0.311 bc | 2.666 ± 0.658 ab | 1.475 ± 0.560 c | 2.687 ± 0.467 ab | 1.604 ± 0.272 c | <0.001 |
| ALA | 18:3 n-3 | 0.130 ± 0.028 bcd | 0.108 ± 0.029 cde | 0.077 ± 0.019 de | 0.076 ± 0.014 e | 0.159 ± 0.061 b | 0.132 ± 0.057 bc | 0.205 ± 0.056 a | 0.101 ± 0.027 bcde | <0.001 |
| GLA | 18:3 n-6 | 0.024 ± 0.005 a | 0.016 ± 0.004 c | 0.016 ± 0.003 c | 0.012 ± 0.003 c | 0.029 ± 0.009 a | 0.018 ± 0.005 bc | 0.022 ± 0.006 ab | 0.016 ± 0.002 c | <0.001 |
| Arachidic | 20:0 | 0.008 ± 0.002 cd | 0.008 ± 0.002 cd | 0.008 ± 0.001 cd | 0.007 ± 0.001 d | 0.014 ± 0.005 a | 0.011 ± 0.002 ab | 0.014 ± 0.002 a | 0.009 ± 0.001 bc | <0.001 |
| Eicosenoic | 20:1 n-9 | 0.054 ± 0.006 cd | 0.048 ± 0.009 de | 0.044 ± 0.007 de | 0.040 ± 0.003 e | 0.083 ± 0.020 a | 0.062 ± 0.009 bc | 0.069 ± 0.009 ab | 0.056 ± 0.006 cd | <0.001 |
| Eicosedienoic | 20:2 n-6 | 0.028 ± 0.007 c | 0.020 ± 0.006 cd | 0.020 ± 0.010 cd | 0.010 ± 0.001 d | 0.064 ± 0.037 a | 0.035 ± 0.016 bc | 0.054 ± 0.016 ab | 0.025 ± 0.008 cd | <0.001 |
| Eicosatrenoic | 20:3 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| DGLA | 20:3 n-6 | 0.021 ± 0.006 bcd | 0.013 ± 0.003 d | 0.012 ± 0.004 d | 0.008 ± 0.001 d | 0.053 ± 0.028 a | 0.03 ± 0.011 bc | 0.034 ± 0.006 b | 0.017 ± 0.003 cd | <0.001 |
| Mead | 20:9 n-9 | 0.006 ± 0.002 cd | 0.005 ± 0.002 cd | 0.004 ± 0.001 d | 0.003 ± 0.001 d | 0.016 ± 0.011 a | 0.012 ± 0.005 ab | 0.01 ± 0.003 bc | 0.006 ± 0.001 cd | <0.001 |
| Arachidonic | 20:4 n-6 | 0.236 ± 0.049 a | 0.159 ± 0.04 bc | 0.197 ± 0.04 ab | 0.139 ± 0.019 cd | 0.241 ± 0.056 a | 0.101 ± 0.029 d | 0.156 ± 0.029 bc | 0.105 ± 0.019 d | <0.001 |
| EPA | 20:5 n-3 | 0.007 ± 0.002 bc | 0.006 ± 0.002 bc | 0.004 ± 0.002 c | 0.005 ± 0.001 c | 0.017 ± 0.009 a | 0.012 ± 0.007 ab | 0.014 ± 0.006 a | 0.007 ± 0.002 bc | <0.001 |
| Behenic | 22:00 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| DTA | 22:4 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |

**Table A6. (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| DPA | 22:5 n-3 | 0.169 ± 0.059 cd | 0.114 ± 0.04 d | 0.085 ± 0.033 d | 0.08 ± 0.015 d | 0.423 ± 0.232 a | 0.267 ± 0.141 bc | 0.316 ± 0.071 ab | 0.155 ± 0.036 cd | <0.001 |
| | 22:5 n-6 | 0.119 ± 0.042 b | 0.072 ± 0.02 bc | 0.064 ± 0.015 bc | 0.043 ± 0.008 c | 0.244 ± 0.15 a | 0.136 ± 0.037 b | 0.137 ± 0.038 b | 0.081 ± 0.025 bc | <0.001 |
| DHA | 22:6 n-3 | 0.199 ± 0.045 cd | 0.114 ± 0.031 d | 0.089 ± 0.023 d | 0.074 ± 0.012 d | 0.538 ± 0.275 a | 0.272 ± 0.093 bc | 0.406 ± 0.049 ab | 0.168 ± 0.043 cd | <0.001 |
| Lignoceric | 24:0 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| Total Cholesterol | | 0.809 ± 0.156 d | 0.931 ± 0.138 cd | 0.990 ± 0.108 bc | 1.176 ± 0.099 a | 1.208 ± 0.164 a | 1.153 ± 0.142 ab | 0.919 ± 0.112 cd | 0.917 ± 0.149 cd | <0.001 |
| Total SFA | | 6.157 ± 1.396 a | 4.66 ± 0.623 bcd | 5.159 ± 0.521 abc | 4.264 ± 0.461 cd | 5.433 ± 0.943 ab | 3.712 ± 0.931 d | 5.17 ± 0.817 abc | 3.89 ± 0.649 d | <0.001 |
| Total MUFA | | 9.532 ± 1.746 a | 6.336 ± 0.612 bc | 7.129 ± 0.761 b | 6.401 ± 0.804 bc | 7.406 ± 1.458 b | 5.134 ± 1.579 c | 7.322 ± 0.87 b | 5.103 ± 0.818 c | <0.001 |
| Total cis-MUFA | | 9.49 ± 1.739 a | 6.3 ± 0.608 bc | 7.096 ± 0.755 b | 6.375 ± 0.802 bc | 7.357 ± 1.441 b | 5.089 ± 1.565 c | 7.268 ± 0.861 b | 5.066 ± 0.814 c | <0.001 |
| total trans-MUFA | | 0.042 ± 0.009 abc | 0.036 ± 0.006 bcd | 0.033 ± 0.008 cd | 0.026 ± 0.005 d | 0.049 ± 0.021 ab | 0.045 ± 0.015 abc | 0.054 ± 0.011 a | 0.037 ± 0.006 bcd | <0.001 |
| Total PUFA | | 4.334 ± 1.141 a | 2.639 ± 0.612 bc | 3.609 ± 1.270 ab | 2.343 ± 0.338 c | 4.43 ± 1.259 a | 2.499 ± 0.897 bc | 4.042 ± 0.598 a | 2.297 ± 0.375 c | <0.001 |
| Total n-6 | | 3.833 ± 1.063 a | 2.296 ± 0.579 bc | 3.343 ± 1.234 a | 2.104 ± 0.328 c | 3.285 ± 0.820 a | 1.796 ± 0.643 c | 3.088 ± 0.502 ab | 1.85 ± 0.306 c | <0.001 |
| Total n-3 | | 0.516 ± 0.140 cd | 0.354 ± 0.138 d | 0.236 ± 0.066 d | 0.234 ± 0.051 d | 1.349 ± 0.661 a | 0.651 ± 0.206 bc | 0.968 ± 0.244 ab | 0.421 ± 0.072 cd | <0.001 |
| n-6:n-3 ratio | | 7.556 ± 1.759 b | 6.402 ± 2.575 bc | 13.924 ± 5.787 a | 8.890 ± 0.926 b | 2.660 ± 2.427 cd | 2.607 ± 0.468 d | 3.223 ± 0.587 d | 4.203 ± 0.976 cd | <0.001 |
| Total OCFA | | 0.055 ± 0.01 ab | 0.045 ± 0.013 bc | 0.045 ± 0.007 bc | 0.037 ± 0.004 c | 0.061 ± 0.013 a | 0.04 ± 0.01 c | 0.059 ± 0.008 a | 0.041 ± 0.007 c | <0.001 |
| Total OBCFA | | 3.768 ± 0.340 ab | 4.111 ± 0.773 a | 3.286 ± 0.145 c | 3.58 ± 0.428 bc | 3.294 ± 0.249 bc | 3.322 ± 0.37 bc | 3.329 ± 0.084 bc | 3.117 ± 0.128 c | <0.001 |
| Total FA | | 20.16 ± 3.931 a | 13.774 ± 1.703 cde | 16.025 ± 1.894 bcd | 13.128 ± 1.482 de | 17.436 ± 3.47 ab | 11.485 ± 3.171 e | 16.692 ± 1.939 bc | 11.427 ± 1.741 e | <0.001 |

**Table A7. Egg yolk fatty acids by month (% of total fatty acids)[1]**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| Caprylic | 8:0 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| Capric | 10:0 | 0.005 ± 0.002 c | 0.010 ± 0.002 ab | 0.010 ± 0.002 bc | 0.012 ± 0.003 a | 0.007 ± 0.004 bc | 0.007 ± 0.001 bc | 0.006 ± 0.001 c | 0.008 ± 0.001 bc | <0.001 |
| Undecanoic | 11:0 | 0.001 ± 0.001 cd | 0.004 ± 0.001 a | 0.003 ± 0.002 ab | 0.004 ± 0.001 a | 0.002 ± 0.002 bc | 0.001 ± 0.001 cd | 0.001 ± 0.000 d | 0.001 ± 0.001 d | <0.001 |
| Lauric | 12:0 | 0.002 ± 0.001 cd | 0.003 ± 0.001 b | 0.004 ± 0.001 ab | 0.005 ± 0.001 a | 0.002 ± 0.001 bc | 0.002 ± 0.000 cd | 0.002 ± 0.000 d | 0.002 ± 0.001 cd | <0.001 |
| Tridecanoic | 13:0 | 0.009 ± 0.001 c | 0.016 ± 0.002 a | 0.014 ± 0.003 a | 0.016 ± 0.005 a | 0.011 ± 0.003 b | 0.011 ± 0.003 bc | 0.010 ± 0.001 bc | 0.011 ± 0.001 bc | <0.001 |
| Myristic | 14:0 | 0.298 ± 0.066 c | 0.442 ± 0.087 a | 0.310 ± 0.056 c | 0.354 ± 0.066 bc | 0.323 ± 0.045 bc | 0.360 ± 0.081 bc | 0.398 ± 0.037 b | 0.32 ± 0.029 c | <0.001 |
| Myristoleic | 14:1 | 0.047 ± 0.014 e | 0.102 ± 0.039 a | 0.047 ± 0.031 d e | 0.079 ± 0.023 abcd | 0.076 ± 0.032 bcde | 0.112 ± 0.037 ab | 0.094 ± 0.008 abc | 0.064 ± 0.016 cde | <0.001 |
| Pentadecanoic | 15:0 | 0.071 ± 0.011 b | 0.080 ± 0.008 a | 0.069 ± 0.006 b | 0.076 ± 0.007 ab | 0.090 ± 0.012 a | 0.078 ± 0.017 ab | 0.093 ± 0.013 a | 0.08 ± 0.01 ab | <0.001 |
| Palmitic | 16:0 | 24.562 ± 1.931 b | 26.844 ± 1.482 a | 25.660 ± 2.528 ab | 25.915 ± 1.182 ab | 25.611 ± 2.946 ab | 26.309 ± 1.942 ab | 24.553 ± 2.531 ab | 26.786 ± 1.685 ab | 0.005 |
| Palmiteladic | 16:1 n-9t | 0.044 ± 0.006 c | 0.050 ± 0.024 c | 0.046 ± 0.009 c | 0.052 ± 0.014 bc | 0.066 ± 0.012 ab | 0.076 ± 0.012 a | 0.077 ± 0.016 a | 0.066 ± 0.016 ab | <0.001 |
| Palmitoleic | 16:1 n-7 | 0.738 ± 0.056 a | 0.566 ± 0.093 bc | 0.597 ± 0.089 b | 0.449 ± 0.095 c | 0.554 ± 0.159 b | 0.567 ± 0.064 bc | 0.561 ± 0.105 bc | 0.652 ± 0.200 ab | <0.001 |
| | 16:1 n-9 | 2.465 ± 0.332 cd | 3.421 ± 0.990 ab | 2.232 ± 0.883 d | 3.141 ± 0.491 abc | 3.112 ± 1.054 bc | 3.991 ± 0.974 a | 3.518 ± 0.336 ab | 3.136 ± 0.812 bc | <0.001 |
| Heptadecanoic | 17:0 | 0.192 ± 0.030 c | 0.206 ± 0.025 bc | 0.196 ± 0.012 c | 0.190 ± 0.024 c | 0.242 ± 0.045 ab | 0.256 ± 0.056 a | 0.244 ± 0.031 ab | 0.260 ± 0.032 a | <0.001 |
| c10-heptadecanoic | 17:1 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| Stearic | 18:0 | 5.283 ± 0.487 c | 6.123 ± 0.677 abc | 6.829 ± 0.593 a | 5.670 ± 1.047 bc | 5.755 ± 0.686 c | 5.668 ± 1.117 bc | 5.348 ± 0.700 c | 6.69 ± 0.888 ab | <0.001 |
| Eladic | 18:1 n-9t | 0.171 ± 0.030 cd | 0.218 ± 0.033 bc | 0.163 ± 0.059 cd | 0.142 ± 0.035 d | 0.214 ± 0.087 bc | 0.294 ± 0.086 a | 0.239 ± 0.038 b | 0.264 ± 0.05 ab | <0.001 |

[1]Means ± standard deviation n = 24 eggs pooled into n = 12 replicates per month [2]Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ $p < 0.05$. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; OCFA, odd-chain fatty acids; OCBFA, odd-chain branched fatty acids; BCFA, branch-chain fatty acids; FA, fatty acids.

**Table A7. (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| Oleic | 18:1 n-9 | 42.227 ± 2.61 ab | 39.433 ± 2.089 bcd | 40.328 ± 2.869 abc | 43.580 ± 1.806 a | 35.698 ± 3.388 e | 36.008 ± 4.656 de | 36.662 ± 4.279 de | 37.911 ± 3.768 cde | <0.001 |
| | 18:1 n-11 | 1.611 ± 0.096 c | 1.746 ± 0.314 bc | 1.156 ± 0.315 d | 1.380 ± 0.162 cd | 2.184 ± 0.804 ab | 2.406 ± 0.378 a | 2.327 ± 0.298 a | 2.044 ± 0.458 ab | <0.001 |
| Linoleic | 18:2 n-6 | 16.502 ± 2.14 ab | 14.578 ± 2.938 bc | 18.653 ± 5.979 a | 14.180 ± 1.866 bc | 15.217 ± 2.840 abc | 12.469 ± 3.610 c | 15.669 ± 2.515 abc | 13.834 ± 1.627 bc | 0.001 |
| CLA | 9c, 11t 18:2 | 0.081 ± 0.012 c | 0.098 ± 0.011 c | 0.073 ± 0.009 c | 0.084 ± 0.015 c | 0.125 ± 0.038 b | 0.150 ± 0.026 a | 0.126 ± 0.010 b | 0.132 ± 0.017 ab | <0.001 |
| | 11t, 13c | 0.045 ± 0.012 d | 0.068 ± 0.008 bc | 0.056 ± 0.005 cd | 0.064 ± 0.016 bcd | 0.076 ± 0.018 bc | 0.090 ± 0.020 a | 0.064 ± 0.009 cd | 0.081 ± 0.010 ab | <0.001 |
| | 11t, 13t | 0.155 ± 0.022 d | 0.206 ± 0.022 cd | 0.158 ± 0.023 d | 0.182 ± 0.042 d | 0.254 ± 0.058 bc | 0.298 ± 0.057 a | 0.236 ± 0.018 bc | 0.268 ± 0.037 ab | <0.001 |
| | t, t | 0.045 ± 0.009 c | 0.071 ± 0.013 ab | 0.059 ± 0.005 bc | 0.064 ± 0.017 abc | 0.062 ± 0.022 abc | 0.081 ± 0.018 a | 0.059 ± 0.009 bc | 0.078 ± 0.006 a | <0.001 |
| ALA | 18:3 n-3 | 0.589 ± 0.144 de | 0.745 ± 0.147 cd | 0.484 ± 0.105 e | 0.598 ± 0.114 de | 0.874 ± 0.353 bc | 1.18 ± 0.249 a | 1.264 ± 0.246 a | 0.966 ± 0.107 b | <0.001 |
| GLA | 18:3 n-6 | 0.12 ± 0.016 bcd | 0.118 ± 0.024 cd | 0.098 ± 0.006 d | 0.096 ± 0.017 d | 0.150 ± 0.034 ab | 0.152 ± 0.027 a | 0.134 ± 0.033 abc | 0.150 ± 0.029 abc | <0.001 |
| Arachidic | 20:0 | 0.042 ± 0.007 e | 0.058 ± 0.007 cd | 0.046 ± 0.006 de | 0.050 ± 0.007 de | 0.074 ± 0.018 bc | 0.098 ± 0.020 a | 0.076 ± 0.009 b | 0.084 ± 0.008 ab | <0.001 |
| Eicosenoic | 20:1 n-9 | 0.274 ± 0.036 d | 0.356 ± 0.040 c d | 0.284 ± 0.02 d | 0.306 ± 0.054 d | 0.434 ± 0.069 b | 0.546 ± 0.101 a | 0.422 ± 0.033 b c | 0.478 ± 0.056 a b | <0.001 |
| Eicosedienoic | 20:2 n-6 | 0.130 ± 0.039 b | 0.144 ± 0.048 b | 0.120 ± 0.053 b | 0.076 ± 0.023 b | 0.332 ± 0.174 a | 0.306 ± 0.092 a | 0.304 ± 0.061 a | 0.225 ± 0.066 a | <0.001 |
| Eicosatrenoic | 20:3 n-3 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| DGLA | 20:3 n-6 | 0.100 ± 0.022 de | 0.104 ± 0.02 de | 0.074 ± 0.030 e | 0.062 ± 0.010 e | 0.334 ± 0.109 a | 0.240 ± 0.045 ab | 0.217 ± 0.049 bc | 0.150 ± 0.027 cd | <0.001 |
| Mead | 20:9 n-9 | 0.030 ± 0.011 cd | 0.035 ± 0.016 cd | 0.027 ± 0.010 d | 0.024 ± 0.009 d | 0.086 ± 0.072 a b | 0.103 ± 0.055 a | 0.058 ± 0.020 bc | 0.048 ± 0.018 cd | <0.001 |
| Arachidonic | 20:4 n-6 | 1.172 ± 0.167 b | 1.180 ± 0.364 b | 1.236 ± 0.171 ab | 1.046 ± 0.163 bc | 1.383 ± 0.333 a | 0.934 ± 0.132 c | 0.907 ± 0.187 c | 0.889 ± 0.223 c | <0.001 |
| EPA | 20:5 n-3 | 0.031 ± 0.003 c | 0.040 ± 0.010 c | 0.026 ± 0.005 c | 0.030 ± 0.008 c | 0.098 ± 0.040 a | 0.086 ± 0.007 a | 0.074 ± 0.019 ab | 0.056 ± 0.014 bc | <0.001 |
| Behenic | 22:00 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |

**Table A7. (cont'd)**

| Fatty Acid | Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| DTA | 22:4 n-6 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| DPA | 22:5 n-3 | 0.869 ± 0.336 cd | 0.876 ± 0.311 cd | 0.487 ± 0.218 d | 0.604 ± 0.165 d | 2.620 ± 1.262 a | 1.954 ± 0.778 a | 1.771 ± 0.324 ab | 1.318 ± 0.428 bc | <0.001 |
| | 22:5 n-6 | 0.638 ± 0.204 bcd | 0.473 ± 0.122 bcd | 0.408 ± 0.06 1 cd | 0.320 ± 0.038 d | 1.412 ± 0.788 a | 1.194 ± 0.305 a | 0.748 ± 0.336 b | 0.724 ± 0.250 bc | <0.001 |
| DHA | 22:6 n-3 | 0.984 ± 0.179 bc | 0.879 ± 0.267 bc | 0.548 ± 0.086 c | 0.567 ± 0.064 c | 3.574 ± 1.401 a | 2.173 ± 0.437 a | 2.455 ± 0.188 a | 1.482 ± 0.352 b | <0.001 |
| Lignoceric | 24:0 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| C14:0-*iso* | 14:0 | LOD | LOD | LOD | LOD | LOD | LOD | LOD | LOD | ND |
| C15:0-*iso* | 15:0 | 0.019 ± 0.005 bc | 0.042 ± 0.019 a | 0.016 ± 0.004 c | 0.023 ± 0.006 bc | 0.019 ± 0.003 bc | 0.022 ± 0.005 b | 0.017 ± 0.003 c | 0.020 ± 0.004 bc | <0.001 |
| C15:0-*anteiso* | 15:0 | 0.010 ± 0.002 c | 0.015 ± 0.002 ab | 0.012 ± 0.003 bc | 0.015 ± 0.004 ab | 0.014 ± 0.005 ab | 0.017 ± 0.004 a | 0.012 ± 0.002 bc | 0.016 ± 0.002 a | <0.001 |
| C16:0-*iso* | 16:0 | 0.048 ± 0.010 c | 0.076 ± 0.013 ab | 0.064 ± 0.009 bc | 0.077 ± 0.016 abc | 0.074 ± 0.027 abc | 0.091 ± 0.022 a | 0.063 ± 0.010 bc | 0.087 ± 0.010 a | <0.001 |
| C17:0-*iso* | 17:0 | 0.064 ± 0.010 d | 0.100 ± 0.030 abc | 0.079 ± 0.012 cd | 0.095 ± 0.022 abcd | 0.092 ± 0.027 bcd | 0.112 ± 0.025 a | 0.079 ± 0.012 cd | 0.107 ± 0.015 ab | <0.001 |
| C17:0-*anteiso* | 17:0 | 0.068 ± 0.014 c | 0.107 ± 0.019 ab | 0.086 ± 0.013 bc | 0.108 ± 0.028 ab | 0.100 ± 0.025 abc | 0.116 ± 0.028 a | 0.084 ± 0.014 bc | 0.110 ± 0.013 a | <0.001 |
| C18:0-*iso* | 18:0 | 0.138 ± 0.034 c | 0.212 ± 0.040 bc | 0.186 ± 0.024 c | 0.211 ± 0.045 bc | 0.199 ± 0.075 bc | 0.272 ± 0.065 a | 0.186 ± 0.027 c | 0.257 ± 0.029 ab | <0.001 |
| C18:0-*anteiso* | 18:0 | 0.139 ± 0.035 c | 0.211 ± 0.039 bc | 0.186 ± 0.023 c | 0.211 ± 0.044 bc | 0.199 ± 0.075 bc | 0.271 ± 0.065 a | 0.184 ± 0.026 c | 0.257 ± 0.028 ab | <0.001 |
| Total SFA | | 30.932 ± 2.591 b | 33.948 ± 2.392 a | 32.904 ± 2.720 ab | 32.740 ± 1.931 ab | 31.767 ± 3.521 ab | 32.701 ± 3.615 ab | 30.889 ± 2.907 b | 34.496 ± 1.425 a | <0.001 |
| Total MUFA | | 47.489 ± 2.413 ab | 46.119 ± 2.659 abc | 45.311 ± 3.698 bc | 49.147 ± 2.236 a | 42.484 ± 1.868 c | 44.094 ± 4.779 bc | 44.136 ± 4.759 bc | 45.288 ± 3.592 bc | <0.001 |

**Table A7. (cont'd)**

| Fatty Acid Carbon Number | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|
| Total cis-MUFA | 47.286 ± 2.343 ab | 45.842 ± 2.605 abc | 45.124 ± 3.654 bc | 48.974 ± 2.261 a | 42.181 ± 1.941 c | 43.761 ± 4.834 bc | 43.792 ± 4.711 bc | 44.980 ± 3.638 bc | <0.001 |
| total trans-MUFA | 0.204 ± 0.041 c d | 0.265 ± 0.042 bcd | 0.210 ± 0.068 c d | 0.192 ± 0.043 d | 0.284 ± 0.079 bc | 0.372 ± 0.112 a | 0.316 ± 0.065 ab | 0.326 ± 0.054 ab | <0.001 |
| Total PUFA | 20.64 ± 2.203 abcd | 19.166 ± 2.089 cd | 21.882 ± 5.833 abc | 17.746 ± 1.839 d | 25.702 ± 2.843 a | 21.752 ± 4.498 abcd | 23.990 ± 2.617 ab | 19.967 ± 2.552 bcd | <0.001 |
| Total n-6 | 18.446 ± 1.972 ab | 16.424 ± 2.322 b | 20.548 ± 6.173 a | 15.875 ± 1.979 b | 18.384 ± 2.209 ab | 15.375 ± 3.820 b | 18.375 ± 2.948 ab | 16.000 ± 1.811 b | <0.001 |
| Total n-3 | 2.448 ± 0.210 b c | 2.598 ± 0.794 bc | 1.568 ± 0.389 c | 1.802 ± 0.280 c | 7.361 ± 2.662 a | 5.401 ± 1.307 a | 5.668 ± 0.417 a | 3.841 ± 0.722 b | <0.001 |
| n-6:n-3 ratio | 7.556 ± 1.759 b | 6.402 ± 2.575 bc | 13.924 ± 5.787 a | 8.890 ± 0.926 b | 2.660 ± 2.427 cd | 2.607 ± 0.468 d | 3.223 ± 0.587 d | 4.203 ± 0.976 cd | <0.001 |
| Total CLA | 0.328 ± 0.050 e | 0.442 ± 0.036 cde | 0.345 ± 0.045 de | 0.390 ± 0.091 cde | 0.488 ± 0.143 bcd | 0.619 ± 0.120 a | 0.486 ± 0.037 bc | 0.557 ± 0.078 ab | <0.001 |
| Total OCFA | 0.162 ± 0.023 d | 0.264 ± 0.070 ab | 0.197 ± 0.034 cd | 0.248 ± 0.061 abc | 0.226 ± 0.058 bcd | 0.266 ± 0.056 a | 0.192 ± 0.032 cd | 0.253 ± 0.033 ab | <0.001 |
| Total OBCFA | 0.273 ± 0.046 b | 0.304 ± 0.032 ab | 0.285 ± 0.018 b | 0.286 ± 0.020 b | 0.346 ± 0.048 a | 0.338 ± 0.074 a | 0.344 ± 0.048 a | 0.348 ± 0.038 a | <0.001 |
| Total BCFA | 0.347 ± 0.065 d | 0.531 ± 0.108 abc | 0.442 ± 0.068 cd | 0.550 ± 0.113 abc | 0.469 ± 0.162 bcd | 0.629 ± 0.151 a | 0.440 ± 0.069 cd | 0.597 ± 0.075 ab | <0.001 |
| Total *iso*BCFA | 0.270 ± 0.050 d | 0.412 ± 0.091 abc | 0.343 ± 0.05 cd | 0.422 ± 0.084 abcd | 0.363 ± 0.143 bcd | 0.496 ± 0.120 a | 0.345 ± 0.053 cd | 0.471 ± 0.058 ab | <0.001 |
| Total *anteiso*BCFA | 0.078 ± 0.015 c | 0.121 ± 0.019 a b | 0.098 ± 0.017 bc | 0.123 ± 0.031 ab | 0.114 ± 0.029 ab | 0.134 ± 0.032 a | 0.096 ± 0.016 bc | 0.126 ± 0.014 a | <0.001 |

**Table A8. Yolk mineral profile of the egg yolks by month[1]**

| Parameter | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | *p*-value[2] |
|---|---|---|---|---|---|---|---|---|---|
| Iron (ug/g FW) | 69.76 ± 7.30 abc | 70.66 ± 5.39 ab | 64.73 ± 6.10 bc | 63.49 ± 5.37 c | 71.90 ± 6.58 a | 66.44 ± 5.02 abc | 66.09 ± 3.51 abc | 63.60 ± 4.00 c | <0.001 |
| Zinc (ug/g FW) | 34.26 ± 1.68 | 34.80 ± 1.44 | 35.46 ± 1.27 | 34.61 ± 1.72 | 33.37 ± 2.27 | 34.77 ± 1.75 | 33.70 ± 1.55 | 34.07 ± 1.47 | 0.073 |
| Copper (ug/g FW) | 1.89 ± 0.14 bc | 1.83 ± 0.11 c | 2.02 ± 0.13 ab | 1.94 ± 0.17 abc | 2.09 ± 0.13 a | 2.09 ± 0.10 a | 1.95 ± 0.09 abc | 1.82 ± 0.13 c | <0.001 |
| Manganese (ug/g FW) | 0.86 ± 0.20 a | 0.70 ± 0.11 ab | 0.70 ± 0.08 ab | 0.58 ± 0.13 b | 0.64 ± 0.17 b | 0.73 ± 0.13 ab | 0.87 ± 0.14 a | 0.69 ± 0.09 b | <0.001 |
| Molybdenum (ug/g FW) | 0.19 ± 0.07 a | 0.17 ± 0.03 ab | 0.12 ± 0.04 b | 0.11 ± 0.03 b | 0.20 ± 0.07 a | 0.16 ± 0.03 ab | 0.13 ± 0.02 b | 0.16 ± 0.03 ab | <0.001 |
| Selenium (ug/g FW) | 0.67 ± 0.13 c | 0.61 ± 0.09 c | 0.57 ± 0.15 c | 0.71 ± 0.14 bc | 1.00 ± 0.15 a | 0.89 ± 0.16 a | 0.87 ± 0.13 ab | 0.87 ± 0.14 ab | <0.001 |
| Calcium (ug/g FW) | 1227.07 ± 69.38 | 1188.78 ± 58.28 | 1247.08 ± 65.68 | 1181.52 ± 68.76 | 1171.15 ± 74.45 | 1197.93 ± 67.25 | 1199.52 ± 74.92 | 1177.54 ± 60.02 | 0.101 |
| Magnesium (ug/g FW) | 115.05 ± 6.65 bc | 116.36 ± 6.50 b | 126.13 ± 6.63 a | 120.11 ± 8.41 ab | 107.66 ± 7.67 c | 115.36 ± 5.27 bc | 116.64 ± 5.68 b | 117.18 ± 7.25 b | <0.001 |
| Potassium (ug/g FW) | 1195.41 ± 69.45 ab | 1191.64 ± 59.40 ab | 1146.69 ± 120.20 b | 1195.46 ± 67.51 ab | 1264.26 ± 88.64 a | 1248.51 ± 61.92 ab | 1155.18 ± 40.16 b | 1207.58 ± 118.01 ab | 0.009 |
| Phosphorus (ug/g FW) | 5444.32 ± 106.85 bcd | 5415.41 ± 39.19 cd | 5574.79 ± 66.39 a | 5511.99 ± 56.71 ab | 5386.93 ± 106.02 d | 5522.30 ± 51.21 ab | 5507.46 ± 78.10 abc | 5552.88 ± 69.01 a | <0.001 |
| Sulfur (ug/g FW) | 1435.44 ± 40.92 | 1409.58 ± 44.86 | 1453.27 ± 39.97 | 1465.40 ± 44.33 | 1412.41 ± 42.94 | 1441.20 ± 50.34 | 1422.17 ± 60.32 | 1441.75 ± 41.34 | 0.046 |
| Sodium (ug/g FW) | 489.01 ± 26.20 abc | 470.05 ± 24.70 bc | 481.38 ± 146.93 abc | 411.69 ± 206.77 c | 530.85 ± 38.14 abc | 561.95 ± 26.09 ab | 579.69 ± 33.69 ab | 605.72 ± 94.76 a | <0.001 |
| Aluminum (ug/g FW) | 1.12 ± 0.25 cd | 1.18 ± 0.18 bcd | 1.31 ± 0.39 bcd | 1.29 ± 0.24 bcd | 1.49 ± 0.25 abc | 1.67 ± 0.24 ab | 1.88 ± 0.91 a | 0.84 ± 0.18 d | <0.001 |

[1]Means ± standard deviation (n = 24 eggs pooled into n = 12 replicates per month) [2]Results of one-way ANOVA. a-e, Means within a row with different letters significantly differ ($p < 0.05$). FW, fresh weight

**Table A9. Yolk PCA Loadings Plot Values[1]**

| Parameter | PC1 | PC2 |
|---|---|---|
| Vitamin E | -0.95147 | -0.29657 |
| Vitamin A | -0.04052 | 0.00976 |
| T. Cholesterol | -0.01465 | -0.00221 |
| T. Carotenoids | -0.21961 | 0.70131 |
| Beta-Carotene | -0.20195 | 0.64473 |
| T. Phenolics | -1.3972e-05 | 7.9297e-05 |
| C18:3 n-3 | -0.00481 | 0.00076 |
| C20:5 n-3 | -8.6264e-05 | 0.00030 |
| C22:5 n-3 | -0.01070 | -0.005764 |
| C22:6 n-3 | -0.01341 | -0.011995 |
| Total SFA | 0.00328 | 0.018644 |
| Total MUFA | 0.02156 | 0.023428 |
| Total PUFA | -0.02276 | -0.046789 |
| Total n-6 | 0.00702 | -0.028988 |
| Total n-3 | -0.02941 | -0.017378 |
| n-6:n-3 ratio | 0.04024 | 0.005900 |

[1]Principal Component Analysis (PCA) loadings for various yolk parameters across two principal components (PC1 and PC2). T. Cholesterol, total cholesterol, T. Carotenoids, total carotenoids, T. Phenolics; total phenolics, C18:3 n-3; ALA, C20:5 n-3; EPA, C22:5 n-3; DPA n-3, C22:6 n-3; DHA, SFA; saturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids, n-6; omega-6 fatty acid, n-3; omega-3 fatty acid

**Table A10. Forage PCA Loadings Plot Values[1]**

| Parameter | PC1 | PC2 |
|---|---|---|
| C18:3 n-3 | 0.07557 | 0.02672 |
| C20:5 n-3 | -0.00084 | -0.00228 |
| C22:5 n-3 | -0.00138 | -0.00399 |
| C22:6 n-3 | -0.00088 | -0.00235 |
| Total SFA | 0.00014 | 0.07726 |
| Total MUFA | -0.03050 | -0.00028 |
| Total PUFA | 0.03036 | -0.07698 |
| Total n-6 | -0.04168 | -0.09586 |
| Total n-3 | 0.07204 | 0.01888 |
| n-6:n-3 ratio | -0.00904 | -0.01958 |
| Vitamin E | 0.01141 | 0.98670 |
| Chlorophyll A | 0.91322 | 0.00301 |
| Chlorophyll B | 0.29565 | -0.06187 |
| T. Carotenoids | 0.25281 | -0.00380 |
| T. Phenolics | 1.9508e-05 | -0.00181 |

[1]Principal Component Analysis (PCA) loadings for various forage parameters across two principal components (PC1 and PC2).C18:3 n-3, alpha-linolenic acid; C20:5 n-3, eicosapentaenoic acid (EPA); C22:5 n-3, docosapentaenoic acid (DPA); C22:6 n-3, docosahexaenoic acid (DHA); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 fatty acids; n-3, omega-3 fatty acids; n-6:n-3 ratio, ratio of omega-6 to omega-3 fatty acids; Vitamin E, tocopherol; T. Carotenoids, total carotenoids; T. Phenolics, total phenolics.