THE EFFECT OF A BOTANICAL EXTRACT BLEND ON BEHAVIOUR, ENRICHMENT USE, STRESS RESPONSE, PRODUCTION PERFORMANCE, AND EGG QUALITY OF LAYING HENS HOUSED IN A FURNISHED CAGE SYSTEM

by

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ABSTRACT

With the phasing of conventional cages in Canada, there are concerns about how birds' affective states may influence their motivation to explore various enrichments. Phytogenic additives have been explored for poultry production and performance due to their health, growth-promoting, antioxidant, anxiolytic and antidepressant activities. The effect of a blend of botanical extracts (Phytozen®Liquid) on the behaviour, enrichment use, stress response, production performance and egg quality of Lohmann Lite hens (38wk old) housed in a furnished cage system was investigated for 16 weeks using a 4-period crossover design. The botanical extract blend lowered feed intake at 38-40wks, increased average daily egg weight at 42-44wks, and enhanced albumen quality throughout the trial. Furthermore, the botanical extract blend improved the use of enrichment objects (perches, scratch mats and nest boxes) and lowered serum corticosterone levels. In conclusion, Phytozen®Liquid has the potential to improve the overall welfare of laying hens in furnished cages.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ADEW = average daily egg weight

ADFI/FI = average daily feed intake/feed intake

BE = botanical extract

FCR = feed conversion ratio

GABA = gamma amino butyric acid

PROC. GLIMMIX – General linear model of mixed procedure

HDEP = hen-day egg production

HU = Haugh unit

SC = serum corticosterone

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CHAPTER 1: Introduction

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1. Introduction

For many years, there has been considerable emphasis on the welfare of egg-laying chickens due to their confinement in cages with restricted room for behavioural expressions (Adeniji, 2012). Contrary to the cage-free (barn, aviaries, and free run) laying hen systems for egg production, the cage system (conventional and enriched housing) is still preferred because it is considered less expensive and more hygienic (Molnár and Szollosi, 2020). However, the conventional cage system has been criticized for two main concerns; a) the limited space allocated per bird and b) the barren nature of the cages. Consequently, increased feather pecking, cannibalism, feather loss, poor plumage, and, most significantly, the restriction on the expression of normal behaviour have posed significant global challenges to hen welfare (Lay et al., 2011). Following the restriction in conventional cage use, enriched housing has been unanimously accepted as a preferred cage type for housing hens. In enriched housing, birds are kept in small groups, with more space per bird (750 cm^2 vs. 550 cm^2) than in conventional cages. The new cage types are furnished with a nest box, perch, and suitable materials for pecking, scratching, and dustbathing behaviours (Rodenburg et al., 2005). In terms of quality of life, furnished cages have a greater inclination and promise of a higher quality of life for laying hens than conventional cages.

Animals must be able to display their natural behaviour regardless of physical or environmental constraints. The Brambell Committee report (Brambell, 1965) included principles for analyzing and implementing animal welfare regulations in intensive livestock production. Surprisingly, the committee report would later become the fulcrum on which the five freedoms were formulated, namely: freedom from a) hunger, thirst, and insufficient food, b) thermal and physical discomfort, c) injuries or diseases, d) fear and chronic stress, and e) freedom to exhibit species-specific behavioural patterns (Mellor, 2016; Webster, 1994). However, the pitfall of the five freedoms model was that it largely focused on the removal of negative states without necessarily the presence of positive.

Consequently, Mellor (2015, 2016, and 2017) later proposed the five domains model of welfare. The five domains model of welfare described animal welfare as the sum of the negative and positive experiences in four functional domains, including nutrition, environment, health, and behaviour, to form the affective state. Over the last two decades, the mainstream consensus has been that good animal welfare requires both the presence of positive experiences (such as pleasure, comfort, and curiosity) and the absence of negative ones (Broom, 1991; Duncan et al., 1989). According to Riber et al. (2018), "environmental enrichment is defined as an improvement of the environment of captive animals, which increases the behavioural opportunities of the animal and leads to improvements of the biological function." Evidence of enhanced biological functioning may include increased lifetime reproductive success, increased fitness and activities, and some expressions of instinctive behaviours. A more stimulating environment could help to alleviate welfare issues in all poultry production systems (Adeniji, 2012; Bailie et al., 2013; Campbell et al., 2019).

Assessing animal welfare is important from the perspectives of animal behaviour and product quality (Soliman and El-Sabrout, 2020). Nutrition and housing can enhance poultry's well-being and help produce high-quality products (Abo Ghanima et al., 2020). A new concept, “healthy chickens – happy chickens,” refer to the interaction between the intrinsic and the extrinsic components of welfare, including health and housing (Rondoni et al., 2020). According to some reports, adding environmental enrichments that promote hens’ exploratory and comfort behaviour positively impacts the animal’s biological functioning and behaviour (Bailie and O’Connell, 2015; Vasdal et al., 2019; Zidar et al., 2018). Consequently, the increasing use of furnished cages necessitates promoting novel strategies to optimize the overall welfare states of laying hens. Optimizing the welfare of laying chickens is based on the understanding that most negative experiences, including discomfort, pain, thirst, sickness, anxiety, fear, and depression, are genetically encoded, and it would require more than just providing a suitable environment with enrichments to permanently neutralize their effects on hen welfare (Mellor, 2016). While the furnished cage systems improve overall bird welfare (Abrahamsson and Tauson, 1997; De Reu et al., 2004; Leyendecker et al., 2005), even the best-planned enrichment programs cannot guarantee that birds will show interest in the objects or use them more consistently due to affective states (Godyń et al., 2019).

Feed additives have been widely used in the poultry industry as a potential replacement for antibiotics growth promoters (AGP). However, despite their efficacy in other animal models, such as broiler chickens (Julien et al., 2021), swine (Brown et al., 2017), and rats (Perveen et al., 2009), their stress mitigating, anxiolytic, and antidepressant potentials in laying hens housed in furnished cages are lacking. Phytogenics are a natural health product explored for their bioactive properties, including antioxidant, growth-promoting, antimicrobial, anxiolytic, and antidepressant activities (Bezabih-Yitbarek, 2015; Rombolà et al., 2017; Zhang and Yao, 2019). At both the scientific and commercial levels, a fuller understanding of how various additives and housing systems can influence laying hen behaviour and performance is critical. There has been no research into the effects of a phytogenic additive blend on the welfare of laying hens kept in furnished housing. Compounds in Phytozen® have been shown to improve mental-well being and promote calm behaviour (Brown et al., 2017; Noirot, 2017; Perveen et al., 2009). This study, therefore, aimed to investigate the effects of Phytozen®Liquid (a phenolic-rich blend of phytogenic additives containing magnesium sulphate, rosemary oil, turmeric, sweet orange, vitamin C, and ginger) on the production performance, enrichment use, behaviour and stress response of Lohmann Lite hens housed in a furnished cage system.

CHAPTER 2: Literature Review

A section of the literature review has been published elsewhere:

Makinde, Taiwo O., and Deborah I. Adewole. 2022. "Can feed additives be used to promote positive behaviour in laying hens? A review." *World's Poultry Science Journal* 78, no. 1: 21-40. Doi:10.1080/00439339.2022.2003171

2.1 Overview of avian housing and welfare

Avian welfare in intensive production has long been a concern for researchers and the public, owing to increased demand for their end products and concern about the housing conditions in which they are raised. The biggest welfare concerns in poultry stem from genetic selection for higher laying rates in laying hens and faster growth rates in meat-type birds (Mack et al., 2013; Meluzzi and Sirri, 2009). Certain management and genetic selection approaches have been used to address the issue of poultry welfare (Hartcher and Jones, 2017; Jones, 2004), but their effectiveness has been judged from the farmer's perspective and not from the birds'. Notably, to meet the basic requirement for biological functioning, welfare regulations must consider birds' perceived demand for a quality life, suggesting that, like all other social animals, birds must have a synergy between their psychological, physiological, and external environments for optimal welfare. Generally, the concept of good welfare in poultry birds emphasizes the need to establish an environment that is stimulating and enriching (Newberry, 1995) to express instinctive behaviour (Mauldin, 1992; Pohle and Cheng, 2009; Wood-Gush and Vestergaard, 1989). Behaviour remains a vital welfare assessment tool for all animals, including laying hens.

Hen housing has evolved considerably from the conventional system to enriched cages and alternative housing systems, such as barns, free-range, and aviaries. The conventional cage was the first attempt to rear laying hens in an environment other than the deep litter system (Widowski et al., 2017). Certain harmful welfare conditions, such as injurious pecking, poor plumage, loss of feathers, and bone problems, may occur due to the limited space given per hen for movement and expression of normal behaviours (Lay et al., 2011). Other behavioural and physiological imbalances connected with poor housing conditions include frustration, boredom, stereotypic behaviours, and elevated plasma corticosterone levels (Karcher and Mench, 2018; Li et al., 2016; Vits et al., 2005). A bird suffering from the conditions listed above has poor welfare. According to Lay et al. (2011), no single housing design is ideal for hen welfare; however, increasing environmental complexity has shown the potential to improve performance and behavioural opportunities, and hence welfare.

Complexities in poultry housing are intended to provide more opportunities for domesticated birds to perform their natural behaviours (Pohle and Cheng, 2009), reduce the frequency of abnormal behaviour (Campbell et al., 2019), and increase bird autonomy over their environment (Marchant-Forde, 2015), as opposed to the barren nature of conventional cages that provide little or no stimulation (Blokhuis et al., 2007). Increased complexity in the poultry environment has been found to decrease fear-related behaviours (Brantsæter et al., 2016; Ellis, 2009; Newberry, 1995), increase activities and occurrences of comfort behaviours such as dustbathing and preening (Pohle and Cheng, 2009; Weeks et al., 2000; Zhao et al., 2014), and decrease leg problems (Bizeray et al., 2002a; Reiter and Bessei, 2001; Tahamtani et al., 2020). Interestingly, several strategies have been deployed to achieve these welfare gains in cages and other alternative systems, including increasing the space allocation per bird (Leone et al., 2007; Nicol, 2015), increasing space between feeders and drinkers (Bizeray et al., 2002b), using small feed particles on litter (Chamove, 1989; Guy and Wright, 2010; Jordan et al., 2011; Newberry, 1995), and adding of manipulable structural materials such as the perches (Appleby et al., 2007; Duncan et al., 1992; Gebhardt-Henrich et al., 2017). Others are nest boxes (Appleby et al., 1992b; Onbąsilar et al., 2015) and pecking objects (Baker et al., 2022; Iqbal et al., 2020). However, complaints arising from hygiene, air quality, cannibalism, and bone problems of aviary-housed hens, such as keel-bone damage (Casey-Trott et al., 2017) continue to favour cage housing for egg production.

Currently, the egg industry in Canada is a fast-growing enterprise, already contributing over 1.3 billion dollars to the country's Gross Domestic Product, or GDP (Egg Farmers of Canada, 2020). In response to the growing public concerns about the welfare of egg-laying chickens, the National Farm Animal Care Council (NFACC, 2017) urged the egg industries to adopt a position that favours improved quality of life for pullets and laying hens, as well as a gradual phase-out of conventional cage use in Canada by 2036. Based on the European Union's successful ban on the use of conventional cages (Pejman et al., 2019), Canada's chances of achieving her objectives are promising. The latest report showed that, between 2016 and 2020, enriched cage utilization increased by 15%, and conventional cage utilization decreased by 20% (Egg Farmers of Canada, 2020).

2.2 Welfare and feelings

The five domain model of welfare recognizes that animal welfare is connected with its affective state or feelings, which is determined by the presence or absence of negative and positive experiences (Mellor, 2017). Due to the subjective nature of feelings, the four functional domains of welfare, namely, nutrition, environment, health, and behaviour, are regarded as sufficient for providing information which could help in assessing their affective states. An animal's feelings are an essential component of its total well-being which could influence its motivation to explore the environment, especially in the presence of strong survival-critical experiences (Broom, 1991; Mellor, 2017). When possible, negative feelings should be identified and avoided. Therefore, according to Melllor (2017), by presenting positive situations in the functional domains of welfare, an animals true feelings or affective state could benefit from predominantly positive states, such as pleasure, comfort, or vitality, while reducing negative states such as fear, hunger, pain or boredom.

2.3 Laying hen Behaviour

Animals’ only way of responding to and interacting with various environmental stimuli is through their behaviour. Stimuli birds interact with includes humans, conspecifics, and their housing. By observing their interactions, we can infer their welfare state (Mattiello et al., 2019; Yeates, 2018). Behaviour is classified into two: Positive and negative (Figure 1). Positive includes social, comfort, and reproductive behaviours, including their different sub-divisions.

Pecking is a social behaviour of all avian species. In conventional cages, where movement is limited, dominant birds peck on the head, feather, or cloaca of lower-ranked birds in the pecking order. Moreover, other causes of feather pecking are multifactorial and may include genetics, feeding management, and nutritional imbalance (Cronin and Glatz, 2020). Fear is a negative behaviour that is associated with negative experiences in their memory (Jones, 1996). Piling is often used to show fear in caged birds, and excessive piling can result in death (Fulton, 2019).

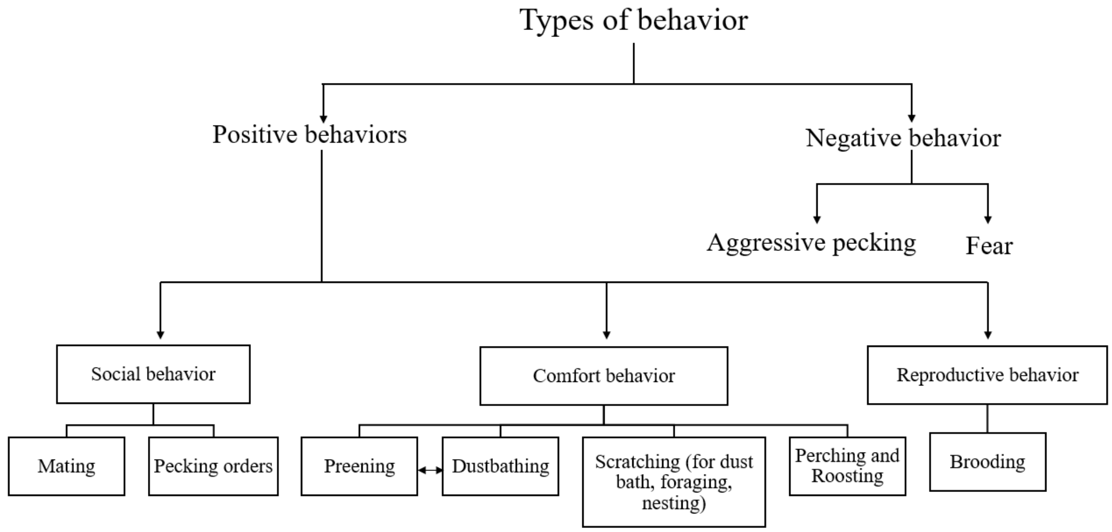

Figure 2.1. Schematic diagram of species-specific behaviour of laying hens

In the presence of a suitable substrate, ground scratching, dustbathing, and preening may occur sequentially. Dustbathing is a comfort behaviour in which poultry birds scoop dust or loose litter materials into their feathers, which helps in controlling ectoparasites and removing oily secretions (Döring, 2013; Faure and Bryan Jones, 1982). Preening is how birds clean or groom their feathers naturally or after dustbathing. Moderate body preening is within the limit of normal behaviour; however, excessive feather preening could be associated with heat stress (Felver-Gant et al., 2012). Perching is a typical behaviour of the wild red jungle fowl. There are controversies about whether the lack of perching behaviour could result in poor welfare. However, the number of birds roosting on perches at one time may indicate the pleasure or comfort chickens gain from it (Pickel et al., 2010), in addition to the common knowledge that it provides a temporary shelter for weak hens fleeing their aggressive mates. Nesting, on the other hand, is a process that begins with pre-laying behaviour and involves hens identifying isolated spots in their habitat. In the wild, laying hens scratch the litter or other substrates of their choice to construct a shape deep enough to hold the eggs together after choosing their favourite nesting spot. Section 2.5 and subsection 2.5.2 fully describe how this behaviour is adapted in furnished cages.

2.4 Impact of stress on poultry

Stress is a physiological and behavioural condition caused by a variety of factors, including environmental changes (Altan et al., 2013; Campbell et al., 2019), transportation (Ghareeb et al., 2008), handling (Nicol, 1992), housing condition and stocking density (Anderson et al., 2021). For instance, heat stress has long posed a significant threat to the welfare of poultry. Heat stress disrupts birds' behaviour and physiological balance (Bhadauria et al., 2014; Daramola et al., 2013). Consequently, poultry species adopt a combination of behavioural, neuroendocrine, and physiological adaptations to maintain thermoregulation at high ambient temperatures (Ilori et al., 2012; Ratnakaran and Madiajagan, 2016; Yahav et al., 2005). A farmed animal is said to be in good welfare if it is comfortable, healthy, well-nourished, safe, and mentally stimulated to express instinctual behaviours (Kelly 2019). According to Jones (1996), stress can cause changes in chicken behaviour, potentially leading to behavioural disorders such as feather pecking and heightened fear reactions. In addition, Nawab et al. (2020) attributed elevated serum corticosterone concentrations in laying hens to heat stress. Stress also impacts other production indices, including shell quality, yolk weight, body pH, and temperature (Janczak and Riber, 2015).

Table 2.1. Ethogram used for behavioural observations

Behavior	**Definition/description**
Forage	Bird pecks or scratches at the wire floor, scratch mat or pecking block
Perching	A hen is roosting on a perch with its two legs for more than 4 seconds (Casey-Trott and Widowski, 2016)
Dustbathing	Hen performs some wing shakes, bill raking, scratching, pecking in plumage (Casey-Trott and Widowski, 2016)
Preening	Hen grooms her wing and feathers with the beak at intervals
Other comfort behaviours	Hens performs feather-ruffling and wing stretching (Casey-Trott and Widowski, 2016)
Gentle feather pecking	Mild pecking at the feathers of conspecific, performed in multiple bouts (Van Hierden et al., 2004b)
Severe feather pecking	Vigorous pecking or pinching at the feathers of a conspecific (Van Hierden et al., 2004)
Sleeping	Hen is relaxed, sitting or standing, with eyes closed. Head may be tucked (Blokhuis, 1984)
Sitting	Hen's body is flush with the bottom of the cage, wings tucked, and head either erect or in a relaxed posture. Eyes are open (Casey-Trott and Widowski, 2016)
Standing	Hen stands on her feet, legs extended, no body movement, but with eyes open (Webster and Hurnik, 1990)

2.5 Environmental Enrichment

Compared to the conventional cages, furnished cages offer greater benefits to poultry welfare. Commonly used enrichments are briefly highlighted below.

2.5.1 Perch structure

Perches enhance domesticated and confined birds' natural roosting behaviour (Hemsworth and Edwards, 2020). Notable contribution of perches to production and welfare of laying hens include reduction in foot damage, increased tibia breaking strength, and reduced feather damage (Appleby et al., 1992a; Duncan et al., 1992; Hu et al., 2021). However, various factors influence perch use, such as perch height, type of material, and bird strain (Faure and Jones, 1982). Variable perch heights in feral birds and caged hens was found to prevent the predation of vulnerable hens (Wood-Gush and Duncan, 1976). According to a related study, Levan et al. (2000) suggested that the use of horizontal and angled perches during hot weather could promote airflow and reduce heat loss in broiler chickens. Perches are also used in alternative systems, such as the aviaries, but they have been connected to keel bone fractures resulting from poor landing (Widowski et al., 2017).

2.5.2 Nest boxes

Nest sites in furnished cages and aviary systems are designed to replicate hens' inherent impulse for nesting in their natural habitat. A private space is commonly delineated by plastic curtains and overlaid with mats or other substrates, or a metal box might be partitioned from the rest of the cage. The nest box has been demonstrated to significantly reduce egg production losses by decreasing cracked eggs and egg-pecking associated with laying in open spaces (Estevez, 2009; Lay et al., 2011).

2.5.3 Scratch mats

The significance of exploratory behaviour cannot be overstated. The more complex an environment is, the more difficult it is to accommodate some behaviour stimulating enrichments, such as exploratory and foraging behaviour. Scratch mat use encourages exploratory behaviour in furnished cages; although, the frequency of this behaviour may be influenced by the presence or absence of litter materials (Sandilands et al., 2021), space allowance (Widowski et al., 2017), and size of the scratch mats (Li et al., 2016). In the alternative systems, the floor is covered with litter.

2.5.4 Pecking objects

Pecking is a natural hen behaviour associated with exploration and foraging. Commercially available pecking blocks could promote exploratory behaviour and decrease agonistic (redirected) behaviours, including feather pecking and cannibalism (Moroki and Tanaka, 2015). It is well acknowledged that the form, colour, size, taste, texture, component, or nutritional composition of the available pecking block influences hens' motivation to peck (Jones et al., 2000). The preference test results in our baseline investigation revealed that when given the choice of more than one block option, hens peck at different block options at varied rates (see supplementary figure 1) (Makinde et al., 2022). Pettersson et al. (2017) earlier reported that providing a resource package reduced feather pecking and boosted exploratory behaviour in a free-range layer farm. The resource package used by Pettersson et al. (2017) included a pecking pan containing a particulate block and wind chimes and a narrow shelter area. Similarly, pecking stones have been shown to reduce feather pecking in non-beak-trimmed birds genetically selected for high pecking behaviour (McAdie et al., 2005; Sherwin et al., 1999; Tahamtani et al., 2016; Van Staaveren et al., 2021).

2.6 Furnished cages

The furnished or enriched cage was designed as an improvement over conventional cages to meet the basic necessity for natural behaviour expression. Due to the provision of varying amounts of space and the incorporation of environmental enrichments, some natural behaviours are now permitted by hens in furnished cages (Appleby et al., 2003). The word "some" is adopted to describe the behaviour expression of laying chickens in enriched cages because of the lack of sufficient stimuli, such as dustbathing, which would force more occurrence of sham dustbathing. As demonstrated in the literature, alternative approaches for replicating this behaviour include using small feed particles on scratch pads (Pokharel et al., 2018). The disadvantage of this method is that the substrates are frequently exhausted and must be replenished (Lay et al., 2011). A furnished cage's size is directly proportional to the size and types of enrichment it can accommodate. A typical enriched cage system includes a nest box, feeder, drinker, various shapes and heights of perches, a scratch pad, and pecking items.

2.7 Current perspectives in environmental enrichment studies

Environmental enrichment is critical to the welfare of poultry. This is evident in its several advantages to poultry birds. However, several animal characteristics like age, bird strain, size and form of enrichment material, poultry kind, and, most importantly, the bird's affective state (emotions, moods) may influence the frequency of enrichment utilization (Godyń et al., 2019). Researchers have generally presumed that enrichment objects have either very appealing or not so attractive attributes that limit how they can be used. The object has always been the focus, while bird "affect" (i.e., mood) has rarely been considered a variable in how birds use enrichment. There is a scarcity of research on how feed additives, including phytogenic plants, can improve enrichment utilization in laying hens by producing a relaxing effect on their brain. The following section examines the overall benefits of feed additives on poultry wellbeing, emphasizing phytogenic additives.

2.8 Feed additives in layer well-being

The use of feed additives on the well-being and performance of poultry has been widely studied because of their antioxidant, growth promoting, immune function, antimicrobial, anti-inflammatory, anxiolytic, and antidepressant activities. Examples of feed additives include prebiotics, probiotics, phytogenic additives, vitamins, minerals, and amino acids. However, concerns relating to regulatory delays, strict storage requirements, and a lack of consistent documentation of its efficacy have limited the commercial application of some non-plant-based additives (Applegate et al., 2010). The use of phytogenic additives in improving bird performance is continuously growing because of ease of accessibility, flexible storage requirement, little or no technical know-how, and economical and safety assurances for both birds and human consumers of poultry products (Krauze et al., 2021).

Phytogenics or botanicals are plant-derived products used extensively to improve poultry's health, growth performance, and welfare (Gabarrou et al., 2017; Julien et al., 2021; Krauze et al., 2021). They are materials from spices, herbal extracts, or plant parts (i.e., rhizomes, leaves, roots, bark, flowers and sometimes, seeds) with a very high accumulation of bioactive substances (Krauze et al., 2021), such as flavonoids, glycosides, saponins, polyphenols, capsaicin, linalool (Grashorn, 2010; Kiczorowska et al., 2015; Windisch et al., 2008). According to Steiner and Syed (2015), phytogenic additives refer to essential oils, spices, herbs, plant extracts, or a combined mix of ingredients that may be added to animal feed in minute quantities to exert their effects.

As earlier established, causes of negative pecking behaviour are multifactorial, and examples include nutritional change or imbalances, stress, the presence of injured birds, overcrowding, and excessive light. Diet change has the potential to initiate feather pecking behaviour in chicks. Though the fundamental mechanism behind this association is currently unexplainable, Dixon et al. (2008) simulated a diet change from a high-quality diet with high protein to a low-quality diet for pullets supplemented with oregano oil. Half of the groups in the diet change treatment with oregano oil supplementation showed the least evidence of dietary neophobia, lessening the incidence of feather pecking in the oregano oil supplemented groups in contrast to the other group that did not receive the essential oil treatment.

2.9 Therapeutic activities of phytogenic additives

2.9.1 Immune regulation, antioxidant, anxiolytic, and antidepressant activities

High temperatures above the comfort zone in birds could result in compromised metabolic and immune system function, an upset to the endocrine system, failure of gut integrity, and tissue damage (Nidamanuri et al., 2017). Propolis supplementation significantly improved standing and walking activities and reduced panting and feather pecking in Ross 308 broiler chicks, Sasso chicks, and ducks reared under heat stress conditions (Omar et al., 2004). This could be due to their antioxidant activities (Prytzyk et al., 2003; Wang et al., 2004). Subsequently, Mahmoud et al. (2015) found that higher levels of propolis supplementation culminated in reduced heat stress responses by increasing walking activities and reduced panting behaviours when graded levels of propolis extract were evaluated on heat-stressed broiler chicks. These findings on propolis extract using the broiler models are some indicators of its anti-oxidant activities. In a related study, the components of propolis (coumarins, amino acids, fatty acids, steroids, inorganic compounds, polyphenols, phenolic aldehydes, phenolic acids, caffeic acids and terpenoids) were shown to enhance glucocorticoid receptor function in the hippocampus, reduce serum corticosterone levels and block the production of reactive oxygen species, thereby limiting stress response and the impact of stress (Hoşnuter et al., 2004).

Recent studies have demonstrated that essential oils from propolis have anxiolytic effects on acutely stressed mouse models by modulating the hypothalamic-pituitary-adrenal (HPA) axis. The repeated administration of *Nigella sativa* (the common black seed) oil on acutely stressed rats produced anxiolytic effects while decreasing serotonin turnover (Perveen et al., 2009). This therapeutic property may be due to thymoquinone, a major bioactive component in *Nigella sativa* oil. The study conducted by Sogut et al. (2008) on broiler chicks using *N. sativa* revealed a decrease in hepatic liver peroxidation and increased activities of several enzymes such as catalase, glutathione-S-transferase, myeloperoxidase and adenosine deaminase. Similarly, thymol is the main phenolic constituent of thyme oil, known to be rich in antioxidants. It demonstrated anxiolytic activities by effectively decreasing fear response and improved feeding behaviour in adult quail hens and broilers (Ramadan, 2013; Lábaque et al., 2013). Krauze *et al.* (2021), on the other hand, fed a phytobiotic preparation containing cinnamon oil and citric acid (in drinking water) to chickens. They found that the chickens improved immunity and growth performance, indicated by the villi lengthening, percentage of phagocytic cells and the antioxidant system indices.

Herbal additives are extracts or plant parts, such as leaves, rhizomes, roots, flowers, bark, or seeds. *Scutellaria baicalensis* L. is an important traditional herb in Chinese medicine. Its root extract, combined with Curcuma extract, has been used in broiler nutrition to decrease gut inflammation-induced heat stress effectively and consequently increase performance (Varmuzova et al., 2015). This action may be due to the presence of wogonin, an active component present in *S. baicalensis*, which was reported to exhibit anxiolytic effects by influencing GABAergic (gamma-Aminobutyric acid) transmission in the b rain in a mice study (Navratilova and Patocka, 2016), which resulted in behavioural changes, especially in stressful situations (Zmrhal et al., 2018). A substance is GABAergic if it modifies the effect of gamma-Aminobutyrate acid in the body or brain by reducing neuronal excitability throughout the nervous system (Froestl, 2011).

2.9.2 Performance, egg production and quality

The increasing awareness of interest in laying hens welfare by consumers and animal welfare groups has made animal welfare an essential factor in market innovations. Current purchasing trends have shown that consumers make informed decisions on eggs produced from their preferred egg production system (Buller and Roe, 2014; Scrinis et al., 2017; Toschi et al., 2013). With the move from conventional to enriched cages, egg quality could be affected by changes in the internal and external structure. Thus, cheap, environmentally friendly veterinary health products could be the key to unlocking and maximizing the welfare of caged hens. There seem to be conflicting reports on the efficacy of phytogenic additives on egg production and quality. The contradictions may be due to a number of factors, including route and age of administration, study duration, plant preparation and bioactive components (Harrington et al., 2020). Hen strains and housing types have also contributed to the inconsistencies (Jones and Anderson, 2013; Pires et al., 2021).

Consequently, there are no monotonous ways to fully establish the productive potentials of phytogenic additives, even though a relationship between phytogenic additives, housing type, hen strain, and age may offer some understanding. Several studies have earlier found that egg quality is affected by housing system and genotype (Ledvinka et al., 2000; Moorthy et al., 2000; Tůmová et al., 2009; Vits et al., 2005; Wall and Tauson, 2002). In addition, the combinative use of phytogenic additives may provide a more robust way to present their applicability to hen welfare.

Egg quality characteristics include albumen quality (height and weight), yolk weight and eggshell characteristics. The industry unit of estimating egg protein quality is the Haugh unit, which is the correlation between egg weight and the albumen height. The higher the Haugh unit score, the higher the quality of the egg white. Egg yolk weight is expected to increase with egg weight. Eggshell characteristics include eggshell weight, thickness, and breaking strength. Egg breaking strength appears to have an inverse relationship with egg weight over the production cycle (Harrington et al., 2020). Feeding dried oregano or rosemary to 28-week-old hens effectively improved shell parameters over 12 weeks (Radwan-Nadia et al., 2008), whereas Bozkurt et al. (2016) did not observe any significant effect of oregano oil on eggshell characteristics of older hens. The addition of 40 mg/kg of cinnamon to the diet of Lohmann LSL-Lite hens kept under cold stress conditions was observed to increase egg production by 16%, and in combination with supplemental levels of zinc, increased egg production by 19% when compared to controls (Torki et al., 2015).

Similarly, a phytogenic blend containing rosemary oil and thyme was reported to improve feed conversion rate (FCR), egg production and egg output in a study that lasted till 52 weeks (Alagawany et al., 2017b). In contrast, a homogenate mixture of raw herbal powders including garlic, marigold, fennel seeds, and thyme fed to 80-week-old Leghorn laying hens for six weeks did not improve egg production and quality parameters, except egg weight (Saki et al., 2014). Furthermore, Ding et al. (2017) found that the effect of an essential oil mix containing thymol and cinnamon on performance characteristics (hen-day egg production and FCR) of Lohmann laying hens was age-dependent.

2.10 Corticosterone

The body's hormonal response to stress involves the activation of the hypothalamic-pituitary (HPA) axis, which in turn triggers the adrenal gland to secret the primary glucocorticoid hormone, corticosterone. Corticosterone is an important biomarker of stress assessment in poultry because of the HPA mechanism (Dou et al., 2019; Mormède et al., 2007; Ramiah et al., 2019; Weimer et al., 2018). In the HPA mechanism, a rise in stress conditions causes the hypothalamus to send hormonal signals to the pituitary gland, which in turn sends a trigger signal to the adrenocorticotropic hormone (ACTH) to stimulate the adrenal gland to release the glucocorticoid hormone, corticosterone (Siege, 1971).

2.11 Components of Phytozen® liquid

The affective state can be positively influenced through the provision of a novel sensory input. Phytogenics or botanicals are plant-derived products that have been used extensively to improve the health, growth performance, and welfare of animals, including poultry (Gabarrou et al., 2017; Julien et al., 2020; Krauze et al., 2021; Viana et al. 2020). The ingredients in Phytozen include magnesium sulphate (1000 mg), rosemary oil (400 mg), turmeric (80 mg), and inactive ingredients sweet orange (*Citrus sinensis*), propylene glycol, vitamin C, and ginger in every 200 ml dose. Therapeutic uses of the components of Phytozen in treating symptoms of anxiety and physical or emotional imbalances have been reported (Alavi et al., 2021; Gupta et al., 2012; Ueki et al., 2014; Julien et al., 2020). According to studies on sweet orange extract, the mode of action of a neurosensory botanical-based additive is by exerting antidepressant effects via smell and taste of the molecules, thereby stimulating parts of the brain involved in emotions (Clouard et al., 2014; and Val-Laillet et al., 2016).

2.11.1 **Magnesium sulphate** (MgSO4)

Nutritional factors may affect laying hens, and dietary mineral and vitamin deficiencies can increase feather-pecking behaviour and cannibalism (Van Krimpen et al., 2008). Yang et al. (2012) evaluated the effects of magnesium sulphate on heat stress-induced oxidative damage. Results revealed that the activities of antioxidative enzymes in broilers were restored in the magnesium sulphate (MgSO4) supplemented groups by preventing stress-induced oxidative damage and improving growth performance in broilers compared to the control. Magnesium is important in body temperature regulation. Rokade et al. (2017) suggested that magnesium sulphate supplementation in heat-stressed broilers will improve performance and welfare. From the experiment by Rokade et al. (2017), serum corticosterone levels decreased significantly in the magnesium sulphate-supplemented groups, as well as a downregulation in the expression of the HSP 70 gene in the jejunal tissues.

2.11.2 Rosemary (*Rosmarinus officinalis*) Oil

Traditional medicine has explored rosemary extracts in therapeutic interventions, with recent studies indicating widespread pharmacological properties. These properties include antioxidative, anti-inflammatory, antinociceptive, anti-depressant, anxiolytic, anti-hysteric, and amelioration of memory and mental fatigue (Alavi et al., 2021; Bakirel et al., 2008; Sasaki et al., 2013; Rahbardar and Hosseinzadeh, 2020). The beneficial medicinal properties of various extracts of rosemary were believed to be due to its constituent carnosic acid, carnosol and rosmarinic acid (Rahbardar and Hosseinzadeh, 2020). Anxiety-like emotional disorders have been linked with chronic levels of stress due to increased activities of the HPA axis and loss of neuronal cells (Rahbardar and Hosseinzadeh, 2020). Rosmarinic and carnosic acid content of *R. officinalis* have been reported to exhibit neurologic functions, including anxiolytic, antidepressive and neuroprotective effects on mice and in vitro neuronal models (Kondo et al., 2015; Sasaki et al., 2013).

Feed conversion ratio, egg weight, and Haugh unit were noted to improve in laying hens supplemented with *R. officinalis* in the diets and significantly reduced the *E. coli* population (Bolukbasi et al., 2008). In a similar study by Cufadar (2018), eggshell quality was improved considerably in hens fed different dietary levels of *R. officinalis* essential oils. In addition, compared to other treatments, rosemary oil supplemented in diets improved hen-day egg production, egg mass, specific gravity, and the number of pores on the shell (Garcia et al., 2019). Furthermore, supplementing rosemary extract in the broiler diet increased antioxidant enzymes, decreased lipid peroxidation levels, and enhanced oxidative stress parameters (Betul et al., 2017).

2.11.3 Turmeric (*Curcuma longa*)

Turmeric, a member of the ginger family (Zingiberaceae), is an herb that has traditionally been shown to possess Curcumin as its main phenolic compound (Pal et al., 2001). Antioxidant, anti-inflammatory, anti-depressant, and neuroprotective functions have also been associated with the herb's pharmacological functions (Chattopadhyay et al., 2004; Gupta et al., 2012). Curcumin supplementation has been shown to have therapeutic potential in reducing cadmium toxicity in mice (Abu-Taweel et al., 2016; Mohajeri et al., 2017; Namgyal et al., 2021), with a significant ameliorative effect on behavioural and biochemical parameters. Curcumin was also reported to improve liver weights and alanine transaminase (ALT) and reduce serum corticosterone and immunoglobulin levels in laying hens exposed to a temperature beyond their thermal comfort zone (Nawab et al., 2020). Varmuzova et al. (2015) earlier reported that turmeric root extract effectively decreased gut inflammation-induced heat stress and consequently increased broilers' performance. Furthermore, Curcumin was said to favour the growth of beneficial bacteria strains such as *Bifidobacteria* and *Lactobacilli*, and significantly reduced the proliferation of pathogenic strains (Mcfadden et al., 2015; Shen et al., 2017).

2.11.4 Sweet Orange (*Citrus sinensis*)

Various essential oil extracts from citrus plants have induced anti-depressive and anxiolytic effects in various animal models (Viana et al., 2020). For instance, studies by Komori et al. (1995) reported that inhalation of volatile components of citrus EO could influence the activity of brain areas such as the hypothalamus, hippocampus, and pyriform. Preclinical and clinical research showed that citrus fragrance could restore the stress-induced cortex and immunosuppression and may have potential antidepressant effects in rats (Costa et al., 2013; Komori et al., 1995). Furthermore, sweet orange peel extract enhances some performance metrics in quails and broiler chickens, including FCR, feed intake and body weight gain (Çiftçi et al., 2016; Ebrahimi et al., 2013), final live weight (Oluremi et al., 2006), increased red blood cell count and improve cold stress tolerance in quail (Ciftci et al., 2016).

2.11.5 **Vitamin C** (Ascorbic acid)

Different studies have shown that vitamin C has immunomodulatory activities. Reviews by Van Hieu et al. (2022) and Ahmadu et al. (2016) comprehensively summarized some of the anti-stress activities of ascorbic acid in mitigating heat stress in poultry by lowering the circulating stress hormone and improving the antioxidant status of chickens, including laying hens. In addition, they reported that vitamin C improved resistance to infectious diseases, reduced body temperature and panting rates of broilers and laying hens. Their summaries also revealed that ascorbic acid supplementation at higher doses improves egg production and external qualities. Furthermore, the review by Hieu et al. (2022) highlighted the therapeutic uses of ascorbic acid as a feed additive in boosting immunity and antioxidant activities of poultry in a heat-stress environment. Similarly, broiler breeders fed fibre and the vitamin C supplemented diet were shown to have improved wing-feather integrity and reduced stereotypic behaviour such as pacing and self-mutilating activities such as excessive grooming and feather pecking (Asensio et al., 2020).

In conclusion, the importance of environmental enrichment to hen welfare cannot be over-emphasized, especially its ability to stimulate normal behaviours in different classes of animals. Therefore, rather than exploring more ways to improve the quality of enrichment, which is still subject to other extrinsic and intrinsic factors, such as genetics, size and type of enrichment material, as well as the physiological status of the birds, it is reasonable to treat poultry birds as social animals with emotions and feelings. It is not new that extracts from plant sources have been used in animal models and human medicine to treat simulated or real-life anxiety and mood disorders; this literature review has identified several plants and their parts that have been used to induce anxiolytic and antidepressant effects in various animal models, with substantial references to their growth enhancement properties. Provision of a blend of phytogenic additives via drinking water could unlock positive emotions and prolonged interest in enrichment use beyond merely serving as antimicrobial growth promoters.

CHAPTER 3: Effects of a botanical extract blend (Phytozen®Liquid) on production performance and egg quality of laying hens housed in a furnished cage system

The first section of the research has been submitted for publication to the Research in Veterinary Science.

Makinde, T.O., J. MacIsaac, and D.I. Adewole. 2022. Effects of a botanical extract blend on production performance and egg quality of laying hens housed in a furnished cage system. Journal of Veterinary Research (submitted).

The work has also been presented at:

2022 PSA Annual Meeting – Poultry Science Association

3. Abstract

Plant extracts are considered natural health products because of their growth-promoting, antioxidant, anti-inflammatory, and antimicrobial properties in poultry. Previous studies have shown that botanical blends could enhance performance metrics of broilers and hens in conventional and organic systems. However, no study has examined the effects of a blend of plant extracts on egg quality and performance characteristics in laying hens housed in furnished cages. This study investigated the effects of Phytozen® Liquid, a commercially formulated blend of botanical extracts (BE), on the performance and egg quality of laying hens in a furnished cage system.

A total of 280 Lohmann Lite laying hens (38-week-old) were randomly allocated to either BE (40 ml Phytozen®Liquid in 200 L of drinking water) or a control treatment group (without BE in drinking water) for 16 weeks (4 × 4-week periods, each period consisting of 3 wk of measurement + 1 wk washout period). Each group was replicated four times with 35 hens/cage. Performance characteristics were recorded daily (feed weight-**FI**, average daily egg weight) and weekly (feed intake, water intake, feed conversion ratio (**FCR**), percentage hen-day egg production-**HDEP**). In contrast, egg quality parameters (eggshell breaking strength, albumen height, albumen weight, yolk weight, and eggshell weight) were measured at the end of each period. Due to the difference in proximity between the top and bottom tiers to light, the tier and tier × treatment interactions were analyzed separately. All data sets were analyzed using the GLIMMIX procedure of Statistical Analysis Software (S.A.S., version 9.4) for repeated measures.

The result of the type III test of fixed effects showed a significant interaction (P<0.01) between BE-supplementation and period for reduced water consumption in periods 2 and 4 (6.95% and 5.62%), higher (P<0.01) average daily egg weight (ADEW) in period 2 (6.34%), and lower (P=0.055) feed intake (ADFI) in Period 1 (4.89%) compared to the control groups. On the other hand, the overall effect of BE supplementation significantly (P<0.05) improved the internal egg qualities, including the albumen height (3.62%) and Haugh unit (1.72%) compared to the control group, irrespective of the period. The BE supplementation did not affect other performance and egg quality parameters measured. There was an overall significant (P<0.05) tier effect on water intake, tier, and treatment effect on ADEW, and tier × treatment interaction on ADFI, FCR, albumen weight, eggshell breaking strength, and egg yolk percentage.

These results suggest that the administration of BE to laying hens up to 52 weeks of age improved some performance and egg quality metrics in furnished cages.

Key words: egg quality, furnished cage, laying hen, botanical extract, production performance

3.1 Introduction

The poultry housing system influences hen performance and egg quality characteristics. Conventional cages ban in the European Union since 2012 have made way for other alternative housing systems for laying chickens. Increasing environmental complexity has positively impacted animal's biological functioning and behavior (Bach et al., 2019; Bailie and O'Connell, 2015; Vasdal et al., 2019; Ventura et al., 2012; Zidar et al., 2018). Even though the most widely accepted alternative housing systems for animal welfare are free-range and aviaries, these rearing systems provide better access to the outdoor range area, and hens are believed to be more comfortable (Denli et al., 2019). However, there are more serious concerns about the impact of these systems on birds and the environment in general. The risk of injury and mortality rates has been shown to predate the free-range and the aviary systems (Casey-Trott et al., 2017; Denli et al., 2019). Consequently, the cage housing system was allowed to remain with some modification to its design, space allowed per bird, and provision of enrichments, known as the furnished cage system. Not only does the furnished cage system provide more space for hens to exhibit normal comfort behavior and reduce harmful behavior, but studies have shown that hens raised in a furnished cage system outperformed conventionally raised hens in terms of performance and egg quality (Harlander-Matauschek et al., 2019; Onbąsilar et al., 2015).

Plant extracts, spices and essential oils are considered natural health products because of their growth-promoting, antioxidant, anti-inflammatory, and antimicrobial properties in poultry (Harris et al., 2021). Plants in the Zingiberaceae family have long been used in traditional oriental medicine and cuisine (Malekizadeh et al., 2012). The combination of garlic and ginger (*Zingiber officinale*) was shown to improve egg weight, hen-day egg production and feed conversion ratio (FCR) of laying hens (Ademola, 2012). The inclusion of ginger in the laying hen diet increased egg production, egg mass and feed intake (Malekizadeh et al., 2012). Similarly, essential oils from peppermint (*Mentha piperita*), lavender (*Lavandula angustifolia*), and spearmint (*Mentha spicata*) were shown to increase FCR and egg production of laying hens due to the presence of phenolic compounds, linalool, linalyl acetate, coumarin, flavonoids and carvone respectively (Torki et al., 2021). Furthermore, the result of supplementing the laying hen diet with fennel seeds, black cumin seeds and hot red pepper improved albumen qualities, yolk index and shell qualities (Abou-Elkhair et al., 2018). In a related study, laying hen fed a phytogenic blend containing fermented *Schisandra chinensis* pomace, fermented *Pinus densiflora* needle extract, and Allium tuberosum powder significantly improved the Haugh unit (Moon et al., 2021).

Phytozen®Liquid contains a blend of rosemary (*Rosmarinus officinalis*) oil, turmeric (*Curcuma longa*), sweet orange (*Citrus sinensis*), and magnesium sulphate. Rosemary is an herb containing rosmarinic acid, camphor, antioxidant carnosol and carnosic acid (Alagawany et al., 2017a). The addition of rosemary essential oil to the diet of laying hens improved feed conversion ratio, egg weight, eggshell quality, and Haugh unit (Bolukbasi et al., 2008; Cufadar, 2018; Garcia et al., 2019). Similarly, turmeric (*Curcuma* *longa*), a member of the ginger family, contains curcumin as its main phenolic compound (Chanda and Ramachandra, 2019). Curcuminoids have a broad spectrum of biological and pharmacological activities, including growth enhancer, antioxidant, anti-inflammatory, antidepressant, and neuroprotective activities, which have been well documented (Dalal et al., 2018; Gupta et al., 2012). Turmeric extract supplementation significantly enhanced egg quality parameters (Liu et al., 2020; Park et al., 2012). Phytochemical screening of sweet orange shows the presence of flavonoids and anthocyanins (Farag et al., 2020). Furthermore, magnesium is associated with improved eggshell quality at higher dietary inclusion levels (Kim et al., 2013).

Despite the beneficial biological properties each plant-derived compound can exhibit, to the authors' best knowledge, no research has examined a blend of these additives on laying hens apart from the few studies addressing the effect of the individual ingredients on laying hen performance and egg quality. Following the results of a previous study on broiler chicks, where the extract blend successfully mitigated the stressful effect of a sudden increase in stocking density (Julien et al., 2021), Phytozen®Liquid may help to consolidate the egg industry's goal of a progressive transition into furnished housing systems. Therefore, this study aimed to determine the effect of a formulated blend of botanical extracts (BE; containing rosemary oil, turmeric, sweet orange extracts, and magnesium sulphate) on production performance and egg quality of laying hens housed in a furnished cage system.

3.2 Materials and Methods

The study was conducted according to the Canadian Council of Animal Care guidelines and approved by the Institutional Ethics Committee of Dalhousie University (protocol code 2020-035; April 2021). The study was conducted from July 15, 2021, until November 3, 2021; the birds used were 38 weeks old at the beginning of this 16-week study (38wks to 53 wks).

3.2.1 Housing

The furnished cage (Specht Ten-Elsen Poultry GmbH, D-47665 Sonsbeck, Germany) was a multi-tier design equipped with wooden perches, nest boxes, scratch mats, and pre-tested pecking blocks (Pecking block, is a commercial product of Probiotech International, St-Hyacinthe, Quebec, Canada). Using a chain, the pecking blocks were attached to one end of each cage. The nest box was fitted at one end of the cage, lined with artificial turf (Astroturf®, 13 × 12 inches with multiple flexible spine clusters), and opened to an egg-collecting belt, with the egg saver wire extending parallel to and underneath the feed trough, 55 cm from the rear partition of the cage to prevent the eggs from falling off the tray. The house was well ventilated and was under a lighting regime of 16 h per day (5 - 10 lux).

3.2.2 Experimental layout and bird management

The experimental design was a 4-period crossover design with four cages/treatment in each period. Each experimental period included three weeks of treatment and one week washout period (no treatment). The BE used in this study is a widely available commercial product (Phytozen®Liquid, by Probiotech International, St-Hyacinthe, Quebec, Canada). As described in Table 3.1, the in-water supplementation of BE was alternated between the top and bottom-tier cages for the length of the trial. Each cage was treated as an experimental unit. A total of 280 Lohmann Lite laying hens were obtained from a reputable commercial farm and were housed in enriched cages. The birds were allocated to 2 treatment groups, each containing four replicate cages of 35 birds, and were subjected to a period of social mixing before the trial. The laying hens diet was formulated to meet or exceed specifications of Lohmann LSL – lite (Lohmann, 2016). The analyzed composition of the diet is presented in Table 3.2. The BE liquid was administered at a recommended dilution of 200 ml in 1000 L of water. Each tier received Phytozen-supplemented water and fresh drinking water as a control and birds assessed them via the nipple drinkers. Feed and water were provided *ad libitum* throughout the experimental period. The water consumption for each experimental unit was monitored using the Paddlewheel Flowmeters (Model RB-400S8-GPM1, F-1000-RT Blue-White Industries).

3.2.3 Production and performance

Feed intake per pen was calculated by the difference between feed offered and orts. Feed intake was expressed on a per pen/hen basis. Data of weekly water intake were used to calculate the mean daily water intake (ml/hen). Eggs were collected, counted, and weighed daily. Daily egg production and total weight per replicate were recorded and expressed on a hen-day basis (Average daily egg weight, **ADEW**). The feed conversion ratio was calculated using grams of feed consumed divided by the average egg mass for each pen per day. There was no mortality; however, weak, and badly pecked birds were removed from the trial and recorded to correct for percentage egg production and feed conversion ratio.

Table 3.1. Experimental layout

Age (Weeks)	Experimental Period	Week #	Group design (Tier)		Measurement
			Top	Bottom	
38	Period 1	Week 1	Phytozen	Control	PP measurements
39		Week 2	Phytozen	Control	PP measurements
40		Week 3	Phytozen	Control	PP and egg quality measurements
41		Week 4	Wash out week		No measurement
42	Period 2	Week 1	Control	Phytozen	PP measurements
43		Week 2	Control	Phytozen	PP measurements
44		Week 3	Control	Phytozen	PP and egg quality measurements
45		Week 4	Wash out week		No measurement
46	Period 3	Week 1	Phytozen	Control	PP measurements
47		Week 2	Phytozen	Control	PP measurements
48		Week 3	Phytozen	Control	PP and egg quality measurements
49		Week 4	Wash out week		No measurement
50	Period 4	Week 1	Control	Phytozen	PP measurements
51		Week 2	Control	Phytozen	PP measurements
52		Week 3	Control	Phytozen	PP and egg quality measurements
53		Week 4	Wash out week		No measurement

Note: Wash out week = no treatment; PP = Production performance

Table 3.2. Ingredient, calculated, and analyzed composition in phase 1 and phase 2 of diet fed to laying hens (as-fed basis, %, unless otherwise stated).

Ingredients	**Laying period (wk)**	
	38 to 41	**42 to 53**
Corn	48.95	52.97
Soybean meal	17.02	13.83
Canola meal	9.96	10.00
Wheat	10.00	10.00
Limestone	4.63	4.57
Soy Oil	2.40	1.82
Shell mix	2.32	2.28
Oyster shell	2.32	2.28
Dicalcium phosphate	1.35	1.23
Vitamin-mineral premix	0.50	0.50
Salt	0.36	0.34
Calculated composition		
Metabolizable energy (Kcal/kg)	2,800	2,800
Crude protein	16.80	15.71
Calcium	3.90	3.82
Available phosphorus	0.42	0.39
Sodium	0.17	0.16
Digestible methionine + cystine	0.58	0.54
DL Methionine premix	0.20	0.17
Digestible lysine	0.73	0.66
Analyzed composition (%)		
Dry matter	88.80	89.53
Crude protein	16.10	16.60
Calcium	3.00	3.91
Potassium	0.61	0.63
Magnesium	0.18	0.18
Total phosphorus	0.49	0.66
Sodium	0.06	0.19
Copper	17.49	29.20
Manganese	70.76	104.84
Zinc	86.64	104.36
Crude fat	5.79	3.59

Vitamin A = 1.00E+09 IU; Vitamin D3, premix = 5.00E+08 IU; Vitamin E = 500000 IU; Vitamin K = 33%; DL Methionine = 0.5kg; DL Ca-pantothenate = 80%; Vitamin B12 = 1000 mg; Niacin

= 99%; Folic Acid = 3%; Choline Chloride = 60%; Biotin = 1000 ppm; Pyridoxine = 99%; Thiamine = 98%; Manganese Oxide = 60%; Zinc Oxide = 72%; Copper Sulfate = 25%; Selenium Premix = 2000 mg; Ethoxyquin = 60%; Wheat middling = 0.5kg

3.2.4 Egg sample collection and analysis

At the third week of each treatment period (weeks 40, 44, 48, and 52), 21 eggs per cage were sampled and analyzed for internal and external egg quality (Albumen height, yolk weight, egg breaking strength and eggshell weight) at the Animal Products and Technology Laboratory, Department of Animal Science and Aquaculture. Each egg was weighed separately and then subjected to egg breaking strength (N) analysis using the T.A.X.T plus texture analyzer (Texture Technologies Corp., Scarsdale, New York, U.S.A.) with Exponential Stable Micro Systems software, version 2.0.7.0. a 30 kg load cell and flat, cylindrical acrylic probe were used to measure the breaking strength. The blunt end of each egg was oriented upwards to apply force. The cracked egg was broken on a flat surface to measure the albumen height afterward. The albumen height was measured using an albumen heigh gauge (OCD™, Technical services and supplies, Chessingham Park, Dunnington, York, England). The yolk was separated from albumen and weighed. The eggshell was weighed with eggshell membranes giving the eggshell weight. The percentage of egg yolk and eggshell was determined by dividing each component's weight over the weight of the whole egg, and the result was multiplied by 100. The weight of the albumen was determined by the difference between the egg weight and the yolk and shell weights. The Haugh unit was calculated as the correlation between albumen height and egg weight (Doyon et al., 1986) using the formula:

$HU = 100 * log\ (h - 1.7W^{0.37} + 7.6)$.

Where:

HU = Haugh Unit

h = Observed height of the albumen (mm)

W = Weight of egg (g)

3.2.5 Statistical analyses

All data sets were analyzed using the GLIMMIX procedure (**PROC GLIMMIX**) of Statistical Analysis Software (SAS., version 9.4) for repeated measures. **PROC UNIVARIATE** was used to assess data distribution prior to analysis, and outlier data were removed. The model included the fixed effects of BE treatment, crossover period, and the resulting interactions using sattherwaithe degrees of freedom, the covariance structure used was autoregressive. The mean and standard errors were calculated using the **LSMEANS** statement for the fixed terms in the mixed models. Results were presented as scatterplots (where there is interaction) and column graphs representing the treatment effects across the four periods. Due to the difference in proximity between the top and bottom tiers to light, the tier and tier × treatment interactions are shown in Table 3.3. Error bars were not shown in the scatterplots because this will negate the constant variance assumption of the error terms. However, the square root of mean square error, √**MSE** (square root of the assumed constant variance of the error terms), was included to indicate the magnitude of variability. A value of $P \leq 0.05$ was considered significant for all measures, and means were separated using Tukey HSD.

3.3 Results and Discussion

According to the literature, poor welfare associated with intensive husbandry may have a negative impact on poultry production results.

3.3.1 *Production performance*

The type III test of fixed effects showed a significant interaction (*P*<0.01) between BE supplementation and period for reduced water consumption, average daily egg weight (*P*<0.01) and feed intake (*P*=0.05) (Figures 1-3). There was an inconsistent effect of the BE blend on water intake, as shown by higher water intake in periods 1 and 3 (6.95% and 5.62%) and lower intake in periods 2 (10.87%) and 4 (5.71%) compared to the control groups. The reason for this inconsistence is not well understood.

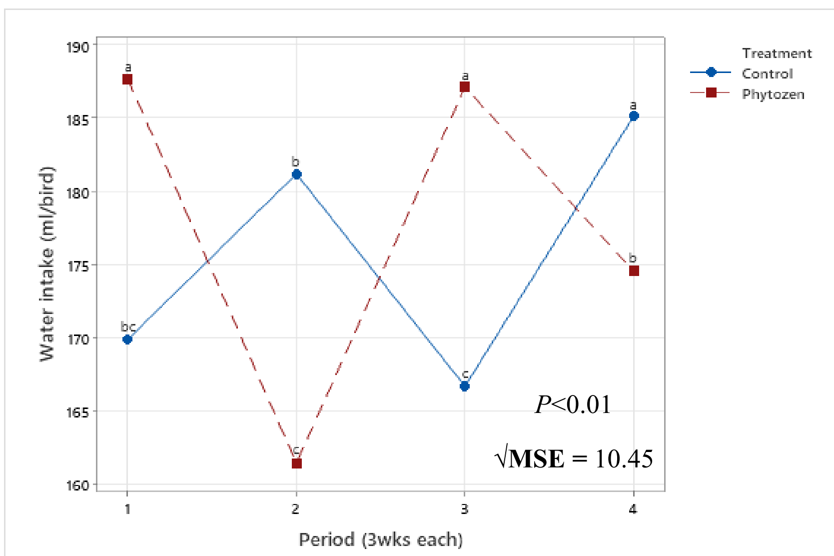

Figure 3.1**.** Effect of BE on water intake (mean ± √MSE) of Lohmann Lite hens. Data represents periodic differences between Phytozen and control groups. Treatments not having similar letter notation are significant (*P*<0.05) from the other.

The average daily egg weight in the BE group was higher in period 2 (6.34%; 42 to 44wks), and feed intake was lower in period 1 (4.89%; 38 to 40wks) compared to the control group. Previous studies report that the dietary supplementation of rosemary oil in laying hen raised from 28 to 76 weeks of age showed improved egg weight and feed intake compared to the control group (Abo-Ghanima et al., 2020). Çiftçi et al. (2016) and Yesilbag et al. (2013) reported similar results for egg weight and feed intake of laying quails (*Coturnix coturnix Japonica* and *Coturnix coturnix Pharoah*) diet supplemented with rosemary oils. However, Alagawany et al. (2017b) did not report a difference in dietary supplementation of an additive blend containing rosemary and thyme herbs on feed intake and egg weight of Hi-sex Brown laying hens. Abo-Ghanima et al. (2020) suggested that a possible mechanism to understand the activity of an essential oil is the promotion of digestive enzyme secretion and gut microflora ecosystem stabilization, leading to enhanced feed utilization.

The factors responsible for the lower feed intake in the botanical extract group from weeks 38 to 40 could be confusing. This is because turmeric and sweet orange have been reported to increase feed intake at dose dependent levels in poultry (Ebrahimi et al. 2013; Wang et al., 2015) due to their antioxidant activities. On the other hand, Malekizadeh et al. (2012) reported that turmeric powder supplemented at 1% decreased feed intake. Therefore, effects of turmeric on feed intake could be due to dose levels in the botanical mix.

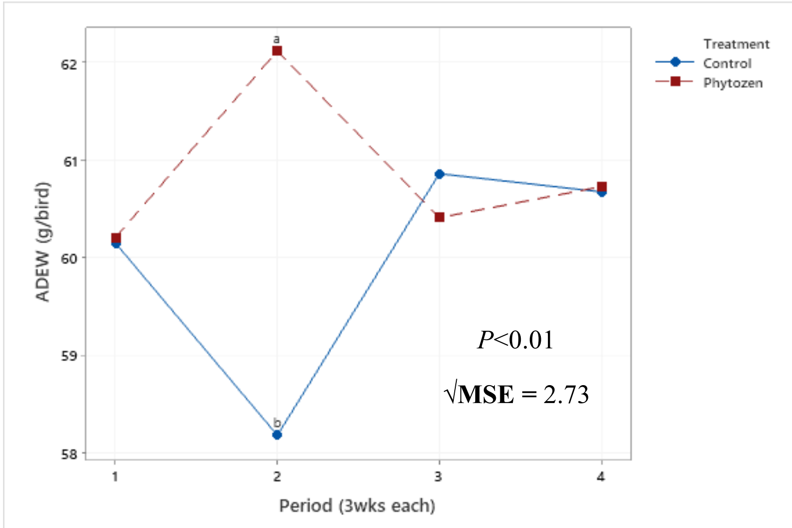

Figure 3.2. Effect of BE on average daily egg weight (mean ± √MSE) of Lohmann Lite hens were measured as a correlation between feed intake and egg weight. Data represents periodic differences between Phytozen and control groups. Treatments not having similar letter notation are significant ($P<0.05$) from the other

Studies by researchers on the use of phytogenic extract blend are not consistent on performance parameters which might be due to the strain of hen and poultry type, duration of experiment, inclusion level of the plant materials, plant parts or material, presence of antinutritional factors, and housing type (Harrington et al., 2020), or an interaction between these factors (Onbaşilar et al., 2018). In this study, BE supplementation did not improve the feed conversion ratio and hen-day egg production (Figures 4 and 5), but a tier versus treatment interaction was recorded for FCR ($P<0.05$) (Table 3). Though water consumption ($P<0.01$) and average daily feed intake ($P=0.01$) were lower in the bottom tier, a higher average daily egg weight ($P<0.01$) was observed in the bottom tier. The observed overall higher water and feed consumption by the top tier hens could be due to their proximity to light (Table 3.3). Supplementary data on egg weight and egg mass (P>0.05) measured at weeks 40, 44, 49, and 52 are presented as figures 3a and b, in the supplementary list of figures.

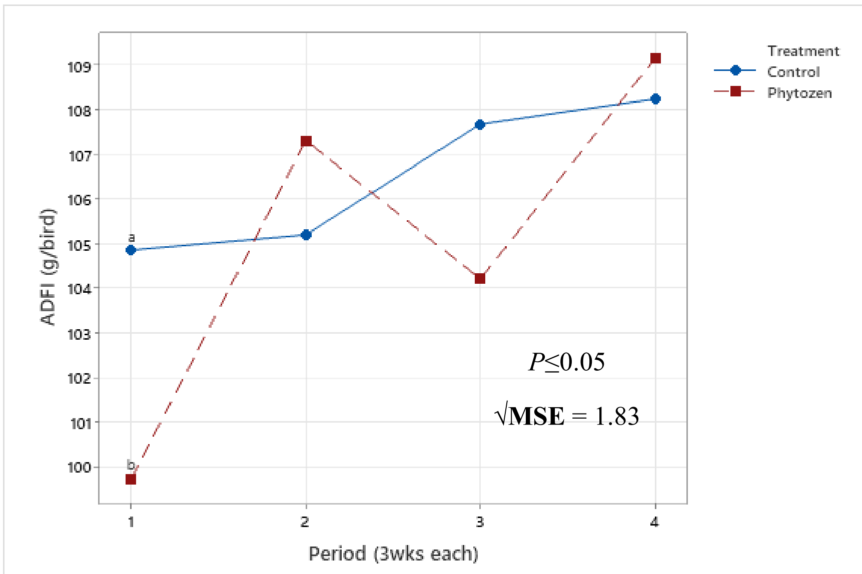

Figure 3.3. Effect of BE on feed intake (mean ± √MSE) of Lohmann Lite hens. Data represents periodic differences between Phytozen and control groups. Treatments not having similar letter notation are significant (*P*<0.05) from the other

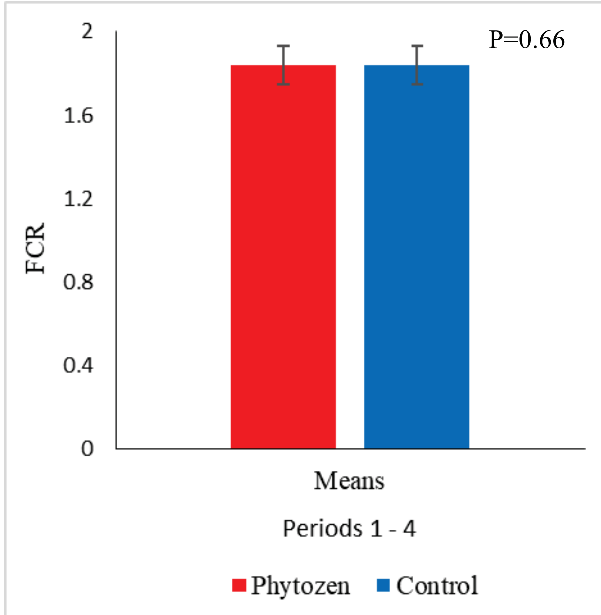

Figure 3.4. Effect of BE on feed conversion ratio of Lohmann Lite hens measured as a correlation between feed intake and egg weight. Values are expressed as means± SE. Data represent overall differences between treatment groups.

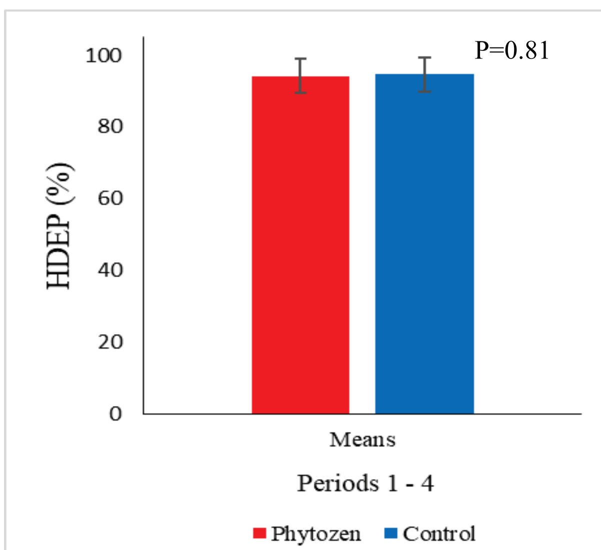

Figure 3.5. Effect of BE on percentage hen-day egg production of Lohmann Lite hens. Values are expressed as means± SE. Data represent overall differences between treatment groups.

3.3.2 *Egg quality*

The effect of in-water supplementation of BE significantly (P<0.05) improved some internal egg quality characteristics, including albumen height and the Haugh unit, by 3.62% and 1.72% compared to the control group, throughout the experimental periods (Figures 3.6 and 3.7). This result could be attributed to the antioxidant activities of the botanical extract blend's bioactive components. However, the BE blend did not affect egg breaking strength, albumen weight, percentage yolk weight and eggshell weight (Figures 3.8 – 3.11). The study conducted by Abo-Ghanima et al. (2020) showed that Haugh unit was significantly improved in the rosemary group compared to the control group. Similarly, dietary supplementation of curcumin in 40 wk old heat-stressed laying hen improved eggshell strength and albumen height in the curcumin group fed for 6 wks (Liu et al., 2020).

Although present as an inactive ingredient in the botanical extract blend, reported anti-oxidant activities of sweet orange may help explain the effects on internal egg quality observed in this study. From 25 to 42 weeks, dried sweet orange peels were found to increase the albumen height and Haugh unit of laying hens (Ahmed et al., 2022).

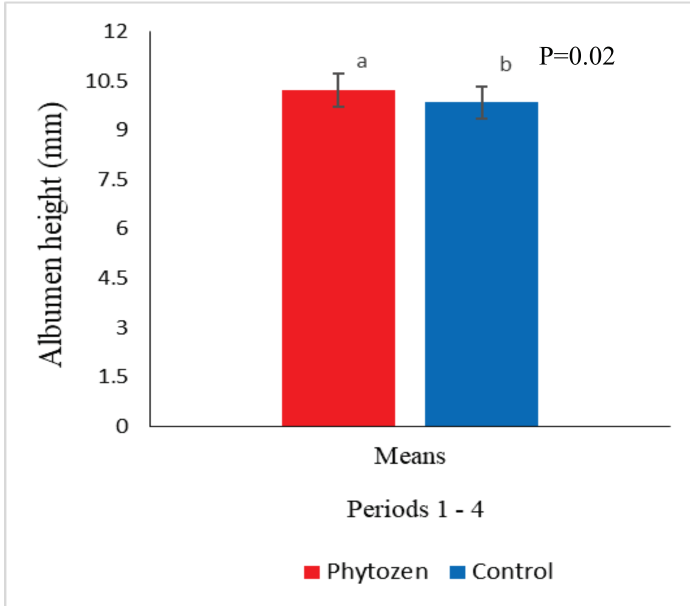

Figure 3.6. Effect of BE on albumen height of Lohmann Lite hens. Values are expressed as means ± SE. Means that do not share similar superscripts differ at $P < 0.05$. Data represent the overall mean difference between treatment groups.

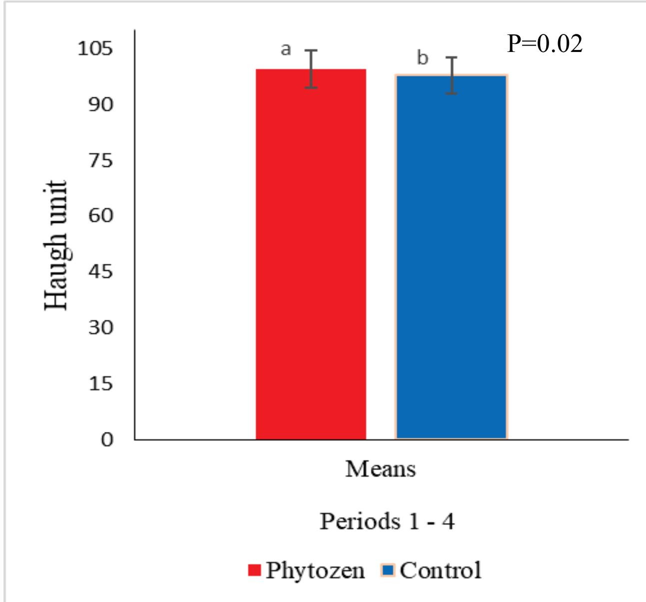

Figure 3.7. Haugh unit of Lohmann Lite hens measured as a correlation between egg weight and albumen height. Values are expressed as means± SE. Means that do not share similar superscripts differ at $P < 0.05$. Data represent the overall mean difference between the treatment groups.

Eggshell mechanical properties are critical for egg safety and economic value (Rossi et al., 2013), as good eggshell quality reduce egg breakage. It was expected that the presence of magnesium sulphate in the BE blend would result in improved eggshell characteristics. However, the eggshell characteristics of Lohmann lite hens were not improved in the current study. At a higher inclusion levels of magnesium sulphate in a water mineral mix, Dushanthi (2015) did not report a significant effect on egg breaking strength, which supports the result in this study. Although, other authors reported that higher dietary levels of magnesium improved eggshell strength in old and young laying hens (Kim et al., 2013; Seo et al., 2010). However, the egg-breaking strength was observed to be higher ($P<0.01$) in the Phytozen supplemented periods in the top tiers than in the Phytozen supplemented periods in the bottom tier (Table 3). Moreover, the tier×treatment interaction was significant for the egg-breaking strength ($P<0.01$), albumen weight ($P<0.02$) and egg yolk percentage ($P=0.03$).

Some studies did not report any positive influence of the bioactive substances from rosemary, turmeric, or sweet orange on performance of laying hens probably due to the rearing duration, dose supplementation, breed, or statistical design (Alagawany et al., 2017; Hassan et al., 2016; Malekizadeh et al., 2012). However, our study clearly shows some evidence of the positive effects of the botanical extract blend The authors attributes the positive effects of the botanical extract to the synergistic action of various active molecules. Furthermore, in a study that compared egg quality of two laying hen strains kept in conventional and enriched housing, Onbaşilar et al. (2018) suggested that improved egg performance may be due to the presence of enrichments. Onbaşilar et al. (2018) concluded that interactions between cage type, strain, and layer age should be considered for egg quality traits. The extent of the interaction between the enriched housing used in this study and the BE blend is unknown. A future study comparing the effect of the BE blend on different hen strains and housing types could be useful in this case. Table 3.4 provides a summary of the effects of phytozen on production and egg quality.

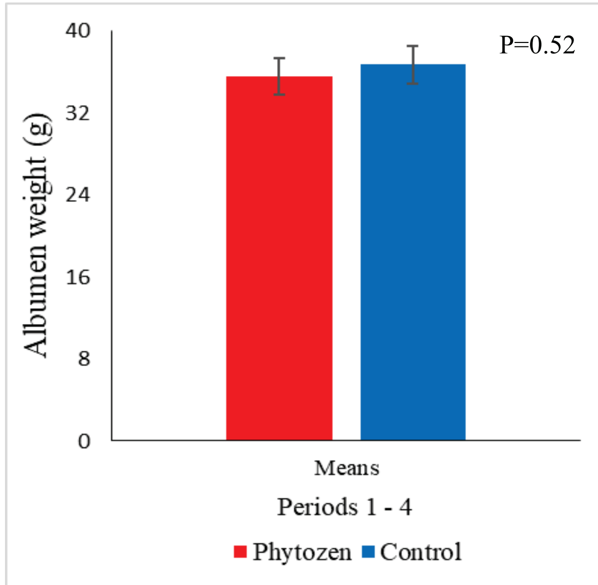

Figure 3.8. Effect of BE on albumen weight of Lohmann Lite hens. Albumen weight is the difference between the egg weight and the yolk and shell weights. Values are expressed as means± SE. Data represent overall differences between treatment groups.

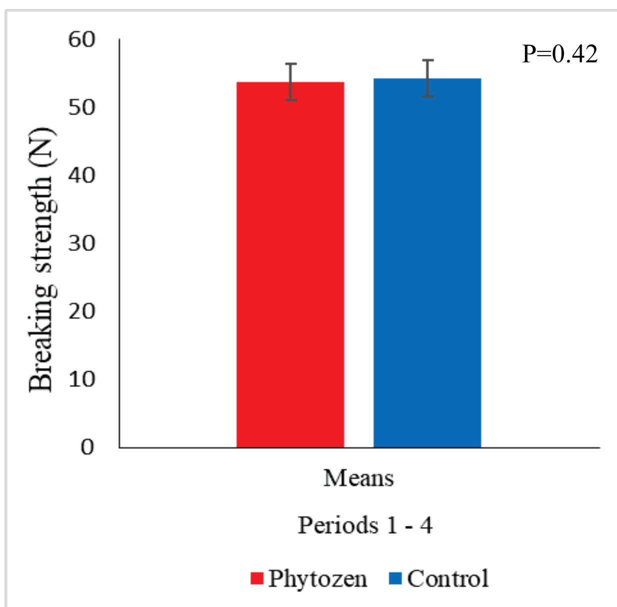

Figure 3.9. Effect of BE on eggshell breaking strength of Lohmann Lite hens. Values are expressed as means ± SE. Data represent overall differences between treatment groups.

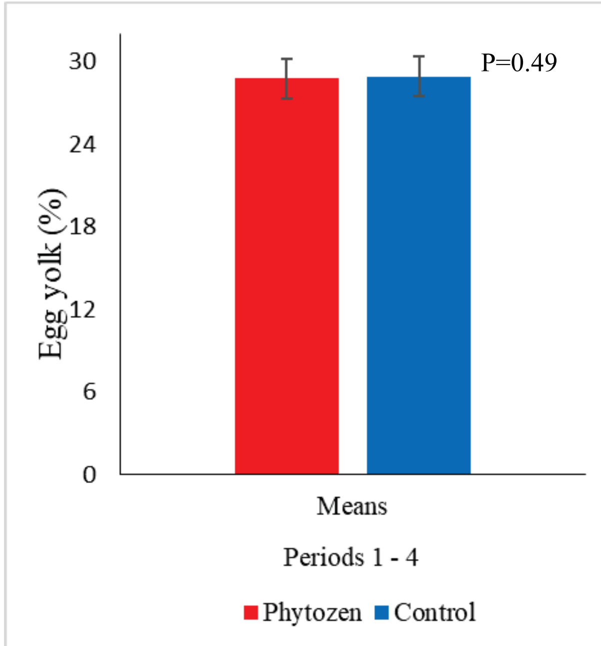

Figure 3.10. Effect of BE on egg yolk weight were measured as a percentage of the egg weight of Lohmann Lite hens. Values are expressed as means± SE. Data represent overall differences between treatment groups.

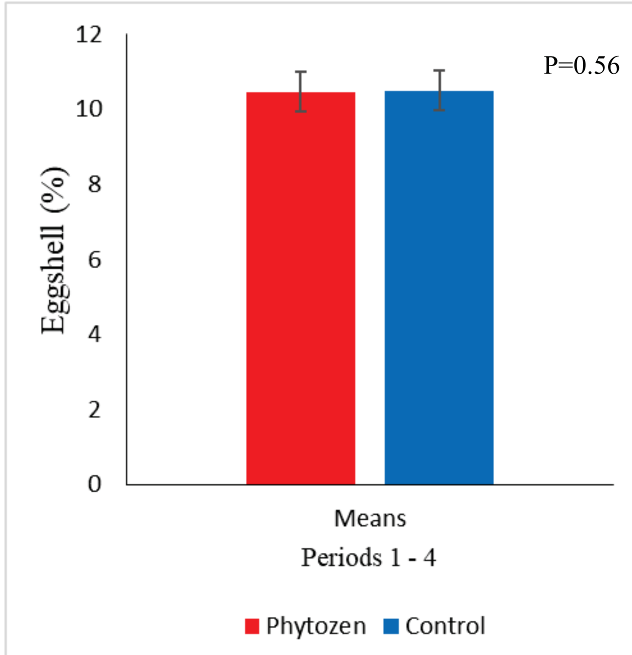

Figure 3.11. Effect of BE on eggshell weight were measured as a percentage of the egg weight of Lohmann Lite hens. Values are expressed as means± SE. Data represent overall differences between treatment groups.

Table 3.3. Tier effect and Tier*treatment interaction of a botanical extract on production performance and egg quality of Lohmann Lite hens housed in a furnished cage system.

Parameter	Treatment					Tier			P-value		
	Tier1 Phytozen	Tier 2 Phytozen	Tier 1 Control	Tier 2 Control	SEM	Tier 1 Overall	Tier 2 Overall	SEM	Trt	Tier	Trt×Tier
Production and Performance											
Water intake (ml/bird/d)	186.32	168.31	179.57	167.38	2.152	182.94^{a}	167.85^{b}	2.25	0.095	< 0.001	0.218
ADEW (g/bird)	60.29	61.35	59.61	60.51	0.372	59.95^{b}	60.93^{a}	0.32	0.057	0.008	0.807
ADFI (g/bird/d)	101.97	108.27	106.74	106.28	1.813	107.27^{a}	104.36^{b}	0.98	0.458	0.011	0.005
FCR	1.827	1.856	1.873	1.804	0.014	1.849	1.830	0.02	0.837	0.259	0.012
HDEP (%)	94.89	94.45	93.86	94.57	1.478	94.38	94.51	2.10	0.763	0.950	0.789
Egg quality											
Albumen height (mm)	10.30	10.19	9.74	10.13	0.163	10.02	10.16	0.21	0.081	0.517	0.253
Haugh unit	100.30	99.37	97.29	99.68	0.769	98.77	99.52	1.06	0.097	0.499	0.131
Albumen weight (g)	35.64	36.88	37.23	35.42	0.308	36.43	36.15	0.52	0.843	0.604	0.017
Egg breaking strength (N)	56.98^{a}	52.61^{c}	53.38^{bc}	56.79^{ab}	0.710	55.18	54.70	0.83	0.689	0.579	0.001
Egg yolk (%)	28.15	28.96	28.94	28.71	0.206	28.54	28.83	0.218	0.212	0.192	0.027
Eggshell (%)	10.48	10.47	10.47	10.55	0.085	10.48	10.51	0.124	0.652	0.765	0.743

Tier 1 (Top cages); the overall effect of the BE extract on the parameters taken on birds from the top cages.

Tier 2 (Bottom cages); the overall effect of the BE extract on the parameters taken on birds from the bottom cage; a, b, $P<0.05$

Note: ADEW, average daily egg weight; ADFI, average daily feed intake; FCR, feed conversion ratio; HDEP, hen-day egg production; SEM, standard error of the mean. Means along the same row with different superscript are significantly different at $P < 0.05$.

Table 3.4. Summary of the Effects of in-water Phytozen supplementation on production performance and egg quality from 38-52 weeks age in laying hens

	Treatment Means		P-value			SEM
	Control	Phytozen	Trt	Period	Trt × Period	
Production performance						
Water intake (ml/bird)	173.55	177.44	0.08	0.01	<0.01	2.09
ADFI (g/bird)	107.28 a	104.87 b	0.14	<0.01	0.05	1.38
ADEW (g/bird)	59.96 b	60.86 a	0.02	0.48	<0.01	0.35
FCR	1.838	1.839	0.95	<0.01	0.24	0.01
HDEP (%)	94.50	95.04	0.81	<0.01	0.68	2.13
Egg quality						
Albumen height (mm)	9.84 a	10.21 b	0.02	<0.01	0.31	0.12
Albumen weight (g)	36.78	36.57	0.52	<0.01	0.81	0.30
Haugh unit	97.89 b	99.60 a	0.02	<0.01	0.52	0.56
Egg breaking strength (N)	54.23	53.70	0.42	<0.01	0.88	0.61
Egg shell (%)	10.49	10.47	0.65	<0.01	0.38	0.06
Egg yolk (%)	28.89	28.75	0.50	0.01	0.39	0.17

Note: ADEW, average daily egg weight; ADFI, average daily feed intake; FCR, feed conversion ratio; HDEP, hen-day egg production; SEM, standard error of the mean. Means along the same row with different superscript are significantly different at $P < 0.05$.

3.4. Conclusions and recommendations

Considering the results obtained in the current study, it could be concluded that the application of this proprietary blend of botanical extracts in the drinking water of Lohmann lite hens showed some impacts on the average daily egg weight and average daily feed intake, and had great effects on albumen qualities. However, except for the egg yolk parameter, all other egg quality metrics decreased with increasing age. We recommend that further studies investigate the use of Phytozen®Liquid with different hen strains and housing systems (including furnished cages, aviary, litter systems and the conventional cage system). In addition, we recommend an early in-water supplementation of the botanical extract blend.

CHAPTER 4: The Effects of a Botanical Extract Blend on Stress Response, Enrichment Use and Behaviour of Laying Hens Housed in a Furnished Cage System

This section of the research has been submitted to the Journal of Applied Animal Behaviour Science.

Makinde, T.O., and Adewole, D.I. 2022. Effects of a botanical extract blend on stress response, enrichment use, and behaviour of laying hens housed in a furnished cage system. Journal of Applied Animal Behaviour Science (Submitted).

4. Abstract

Phytogenic additives have been used in the treatment of anxiety and physical and emotional imbalances because of their antioxidant, anxiolytic, and antidepressant activities. However, no study has investigated the effects of a blend of plant extracts on the stress response, environmental enrichment use, and behaviour of laying hens housed in furnished cages. This study examined the effects of Phytozen® Liquid, a commercially formulated blend of botanical extracts (BE), on the stress response, enrichment use and behaviour of laying hens in a furnished cage system.

280 Lohmann Lite hens (38-week-old) were randomly allocated to either BE (40 ml Phytozen® Liquid in 200 L of drinking water) or a control treatment group (without BE in drinking water) for 16 weeks (4 x 4-week periods, each period consisting of 3 wk of measurement + 1 wk washout period) in a multi-tier furnished cage system. Each group had four furnished cages with 35 hens/cage. At 40, 44, 48, and 52 wks of age, blood samples from 5 birds per cage were taken from the brachial vein, while scan samples from daily video recordings were obtained to analyze for enrichment use and behaviour,

and pecking blocks were weighed weekly. All data sets were analyzed using the GLIMMIX procedure of Statistical Analysis Software (S.A.S., version 9.4) for repeated measures.

The fixed effect of the BE supplementation showed a reduced (*P*=0.02) serum corticosterone (**SC**) concentration (18.00% lower) compared to the control over time, irrespective of the periods. A significant interaction (*P*=0.04) between birds' age and BE supplementation was observed for perch use by hens in the afternoon. In period 3 (46-48wk), the BE supplemented hens used the perches 2.06% more in the afternoon than in the control groups. However, no significance was observed between the treatments for perch use in the morning. Similarly, the BE supplementation improved (*P*<0.01) scratch mat usage in the afternoon by 6.44% compared to the control throughout the trial. The BE increased (*P*<0.01) nest box use in the morning and afternoon compared to the control groups (11.37% and 15.10%, respectively), irrespective of the period or age. However, BE supplementation did not affect hens' pecking behaviour, pecking block usage, preening, and injurious pecking (*P*>0.05), irrespective of time of day and period. Significant tier-treatment differences were observed for serum corticosterone levels, perches, scratch mats, and pecking block use.

The result of the physiological response and enrichment use by Lohmann Lite hens housed in a furnished cage system showed that in-water supplementation of BE extract could improve laying hens' positive affective state and overall quality of life.

Keywords: Environmental enrichment, furnished cages, crossover periods, scan sampling, welfare, botanical extracts

4.1 Introduction

Many countries have banned the use of conventional cages in a bid to enhance the quality of life for laying hens. Since 2012, the furnished cage housing remains the only cage system permitted for keeping laying hens in the European Union (EU). Similarly, the National Farm Animal Care Council's (NFACC, 2017) Canadian Codes of Practice for Pullets and Laying hens advised all egg farmers to embrace all initiatives to improve the quality of life of laying hens, including the gradual phase-out of conventional cages in Canada. Furnished housing offer the benefits of small group size in cages while reducing the poor air quality and poor hygiene that are sometimes associated with aviary systems (Tauson, 2002). Furthermore, the enriched housing system increases space allocation per bird compared to the conventional cages (Leone et al., 2007; Nicol, 2015; Rodenburg et al., 2005) and provides resources such as perches, nest boxes, scratch mats, and pecking objects that satisfy some behavioural needs of laying hens (Lukanov and Alexieva, 2013; Nicol, 2015). Besides increasing the opportunity for performing some instinctive behaviours, furnished housing also contributes to reducing the frequency of abnormal behaviour (Hartcher and Jones, 2017), increases the autonomy of birds over their environment (Marchant-Forde, 2015), reduces fear-related behaviours (Brantsæter et al., 2016; Ellis, 2009; Newberry, 1995), increases activity (Bach et al., 2019), and decreases bone fragility (Bizeray et al., 2002; Reiter and Bessei, 2001).

According to research on rats (Brydges et al., 2011; Harding et al., 2004), and laying hens (Campbell et al., 2019), animals housed in enriched housing have better welfare and are thus presumed to have a more positive experience than those reared in barren environments (Douglas et al., 2012). Even though enrichment materials are provided, there is no guarantee that birds will be interested in them or use them frequently (Godyń et al., 2019). This is logical given that a variety of factors, including age, strain, enrichment material size and shape, poultry type, and the bird's affective states (emotions, moods, and affect) can all influence enrichment utilisation. While measurable physiological factors in birds can be manipulated to maintain homeostasis, the existence of enrichment structures increases the complexity of the environment and allows the birds to exercise more autonomy over their environment. The affective state, on the other hand, is a measurement of bird's mood and emotion, which can include anger, pain, frustration, pleasure, or comfort (Marchant-Forde, 2015). Several study publications provide ample evidence that poultry species have a limited interest in various enrichment items due to their affective state (Jones, 2004; Pichova et al., 2016; Riber et al., 2018). As environmental enrichment (EE) research advances, more focus is needed on strategies for improving and sustaining positive and repeated experiences in intensive production systems (Boissy et al., 2007; Vigors, 2019). Positive and repeated experience are expected to promote good welfare in general (Burgdorf and Panksepp, 2006; Désiré et al., 2002; Yeates and Main, 2008).

Furthermore, EE could potentially alter animal's physiology and behaviour in a variety of ways that have an impact on their physical and mental well-being (Pohle and Cheng, 2009). Corticosterone is an important hormone in the regulation of stress responses to environmental enrichment in animals, including laying hens (Bari et al 2020; Campbell et al., 2020). There are debates on the extent of the effects of EE on corticosterone level. Fox et al. (2006) suggested that EE could decrease emotional reactivity by lowering stress hormone levels, including corticosterone. The second area of debate is the effect of EE on resting corticosterone levels. Reports on studies involving turkeys and male mice alluded to the presence of novel objects and an increase in male aggression as potential sources of ongoing mild stress in enriched environments (Huff et al., 2003; Marashi et al., 2003).

Phytogenic additives have been demonstrated to be efficient in mitigating stress, anxiety, and emotional disorders in broiler chickens (Julien et al., 2021), swine (Brown et al., 2017) and rats (Perveen et al., 2009). As the poultry industry embraces the complexity of furnished cage housing for laying hens, innovative approaches for a positive emotional state must be promoted. The utilisation of botanical extracts is an innovative strategy. A recent review (Makinde and Adewole, 2022) showed that feed additives could help promote positive behaviour in laying hens, enhancing their quality of life across different housing systems. Animal studies have demonstrated that rosemary, turmeric, sweet orange, and magnesium sulphate have a variety of therapeutic effects. These properties include antioxidative (Alavi et al., 2021; Bakirel et al., 2008), antidepressant (Chattopadhyay et al., 2004; Poleszak et al., 2004; Sasaki et al., 2013), mental fatigue alleviation (Abu-Taweel, 2016; Heinrich et al., 2006), cognitive performance and mood improvement (Moss et al., 2009), and neuroprotective activities (Gupta et al., 2012; Sasaki et al., 2013). The bioactive compounds in rosemary oil (α-pinene and rosmarinic acid: Alagawany et al., 2017a; Villareal et al., 2017), turmeric (curcumin: Ceremuga et al., 2017), sweet orange (limonene: Lima et al., 2013), and ascorbic acid (Fraga et al., 2018) could be responsible for the blend's anxiolytic and antidepressant properties.

A blend of phytogenic additives, containing essential oils, has been shown to stimulate areas of the brain related with smell and taste (amygdala, insular cortex and prepyriform), resulting in antidepressant effects (Ceremuga et al., 2017; Clouard et al., 2015; Pant et al., 2019; Val-Laillet et al., 2016; Villareal et al., 2017). Similarly, plant extracts such as rosemary oil, turmeric, sweet orange, and magnesium sulphate have been shown to lower circulating stress hormones in laying hens and broiler chicks (Mack et al., 2013; Nawab et al., 2020; Quinteiro-Filho et al., 2012). As a result, it is hypothesized that a fusion of these easily accessible and available plant derivatives would promote positive behaviour, thereby enhancing laying hen enrichment use and overall welfare performance. Earlier, Julien et al. (2021) showed that Phytozen effectively attenuated the effects of a sudden high stocking density on broiler chicks. In Chapter 3, we have shown that a blend of botanical extracts (BE) containing rosemary oil, turmeric, sweet orange extracts, and magnesium sulphate) improved some production performance and egg quality of Lohmann Lite hens housed in a furnished cage system (Makinde et al., 2022); therefore, this study investigates the effect of BE on the stress response, enrichment use, and behaviour of laying hens housed in a furnished cage system.

4.2 Methods

The study was conducted according to the Canadian Council of Animal Care guidelines and approved by the Institutional Ethics Committee of Dalhousie University (protocol code 2020-035; April 2021). In addition, the housing, bird management and experimental design are similar to what was reported by Makinde et al. (2022).

4.2.1 Housing

The furnished cage (Specht Ten-Elsen Poultry GmbH, D-47665 Sonsbeck, Germany) was a multi-tier design equipped with wooden perches, nest boxes, scratch mats, and pre-tested pecking blocks (Figure 1: Supplementary figures). The pecking blocks were attached to one end of each cage using a chain. The nest box was fitted at one end of the cage, lined with artificial turf (33.2 × 30.5 cm with multiple flexible spine clusters—same material as the scratch mats, Astroturf®), and opened to an egg-collecting belt, with the egg saver wire extending parallel to and underneath the feed trough, 55 cm from the rear partition of the cage to prevent the eggs from falling off the tray. The furnished cages were located in a ventilated room with standard temperature between 21 to 27ºC and under a 16h/d lighting regime (5 - 10 lux).

4.2.2 Bird management

A total of 280 38-week-old Lohmann Lite laying hens were obtained from a reputable commercial farm and were housed in furnished cages. The birds were allocated to 2 treatment groups, each containing four replicate cages of 35 birds, and were subjected to a period of social mixing before the trial. The experiment started at 38 weeks of age and lasted to 53 weeks. The laying hens diet was formulated to meet or exceed specifications of Lohmann LSL – lite (Lohmann, 2016). Please see Table 3.2 of chapter three for the analyzed composition of the 2-phase diet fed to the Lohmann Lite laying hen. Feed and water were provided *ad libitum* throughout the experimental period. The water consumption for each experimental unit was monitored using the Paddlewheel Flowmeters (Model RB-400S8-GPM1, F-1000-RT Blue-White Industries).

4.2.3 Experimental layout and botanical extract source

The experimental design was a 4-period crossover design with four cages/treatment in each period. Each experimental period included three weeks of treatment and one week washout period (no treatment and no measurement). The BE used in this study is a widely available commercial product (Phytozen® Liquid, by Probiotech International Inc., St-Hyacinthe, Quebec, Canada). The BE liquid was administered at a recommended dilution of 200 ml in 1000 L of water. Each tier received Phytozen-supplemented water and fresh drinking water as a control and birds assessed them via the nipple drinkers. As described in Table 4.1, the in-water supplementation of BE was alternated between the top and bottom-tier cages for the length of the trial. Each cage is treated as an experimental unit.

4.2.4 Enrichment use and behaviour sampling

Two video cameras (Sensor type: 1/3 inch Type CMOS Sensor; Sensor pixels 1920×1080) were installed on all four replicate cages of each treatment. Daily video recordings of enrichment use and behaviour of laying hens were done in the morning (8:30 am – 11:30 am) and afternoon (14:00 pm – 17:00 pm) using the Network Video Recorder (Model 6499-24 Digital Video Recorder) throughout the treatment periods, from which only three days (Mondays, Wednesdays and Saturdays) of recordings were analyzed by 10 minutes scan sampling. The enrichment use and behaviour performed were analyzed separately for both times of day (morning and afternoon) following the method prescribed by Li et al. (2016) and Pereira et al. (2007). The pecking block (PB) use was analyzed as both a percentage of birds seen pecking on the enrichment in each time scan (pecking behaviour), and the average weekly weight in grams of PB use per bird. Laying hen's enrichment use, including perches, nest boxes, and scratch mats, were categorized as state behaviours, whereas pecking behaviour, preening, and injurious pecking are event behaviours. The behavioural category definitions are presented in Table 4.2. Thirty-six scan samples of each state and event behaviours were collected per camera daily for the eight experimental units. A total of 10,368 scans were obtained. Nine birds with badly pecked feet were removed from the flock during the trial. Egg production was calculated on a hen-day basis for the eggs collected from each experimental unit.

4.2.5 Serum corticosterone (SC) analysis

Blood samples (2 ml) were collected from the brachial vein using 23-gauge needle into serum tubes from 5 birds in each experimental unit at 40, 44, 48, and 52 weeks of age, and the birds were leg-tagged. Blood collection method followed the method of Littin and Cockrem (2001). All samples were taken within 3 minutes of the birds being removed from the cage and blood collection to minimize the effects of sampling on serum corticosterone. All blood samples were allowed to coagulate for 2 hrs at room temperature and centrifuged at 2,500 rpm for 10 mins (22ºC). The serum was aliquoted and frozen at -20 ºC until analysis. The serum corticosterone (**SC**) was measured by DetectX Corticosterone EIA. (Arbor Assays, Ann Arbor, MI). All samples were analyzed in duplicate. The sensitivity of the assay was 20.9 pg/ mL, with a detection limit of 17.5 pg/mL.

As reported by Arbor Assay, the cross-reactivities of the assay was reported for corticosterone (100%), corticosterone-21-hemisuccinate (18.90%), cortisone (12.3%), estradiol (3.3%), 17 hydroxyprogesterone (2.44%), allopregnanolone (0.76), Dehydroepiandrosterone sulphate (0.62%), estrone-3-glucuronide (0.38), and estrone-3-sulphate (0.24%). The optical density was generated by reading the well in a microtiter plate reader at 450 nm using the KC4 (Kineticalc for Windows, Bio-Tek Instruments, Inc.). The SC concentration of each sample was calculated using the 4PL software on MyAssays after correcting for the dilution of the sample.

4.2.6 Statistical analyses

All data sets were analyzed using the GLIMMIX procedure (**PROC GLIMMIX**) of Statistical Analysis Software (S.A.S., version 9.4) for repeated measures. The model included the fixed effects of BE treatment, crossover period, and the resulting interactions using sattherwaithe degree of freedom, covariance structure used was autoregressive. Both the state and event behaviours were presented as a percentage of the total observations for morning and afternoon. The mean and standard errors were calculated using the **LSMEANS** statement for the fixed terms in the mixed models. Results were presented as scatterplots (where there is interaction) and column graphs representing the treatment effects across the four periods. Due to the difference in proximity between the top and bottom tiers to light, the tier and tier × treatment interactions are shown in Table 4.3. Error bars were not shown in the scatterplots because this will negate the constant variance assumption of the error terms. However, to indicate the magnitude of variability, the square root of mean square error was added, √**MSE** (square root of the assumed constant variance of the error terms). **PROC UNIVARIATE** was used to assess the distribution of data prior to analysis. Natural logarithm transformation test was performed for non-normal data. A value of $P \leq 0.05$ was considered significant for all measures, and means were separated using Tukey HSD.

Table 4.1. Experimental layout

Age (Weeks)	Experimental Period	Week #	Group design (Tier)		Measurement
			Top	Bottom	
38	Period 1	Week 1	Phytozen	Control	VR and weighing of pecking block
39		Week 2	Phytozen	Control	VR and weighing of pecking block
40		Week 3	Phytozen	Control	VR, weighing of pecking block and blood sampling
41		Week 4	Wash out week		No measurement
42	Period 2	Week 1	Control	Phytozen	VR and weighing of pecking block
43		Week 2	Control	Phytozen	VR and weighing of pecking block
44		Week 3	Control	Phytozen	VR, weighing of pecking block and blood sampling
45		Week 4	Wash out week		No measurement
46	Period 3	Week 1	Phytozen	Control	VR and weighing of pecking block
47		Week 2	Phytozen	Control	VR and weighing of pecking block
48		Week 3	Phytozen	Control	VR, weighing of pecking block and blood sampling
49		Week 4	Wash out week		No measurement
50	Period 4	Week 1	Control	Phytozen	VR and weighing of pecking block
51		Week 2	Control	Phytozen	VR and weighing of pecking block
52		Week 3	Control	Phytozen	VR, weighing of pecking block and blood sampling
53		Week 4	Wash out week		No measurement

Wash out week = no treatment; VR = video recording

Table 4.1. Ethogram of enrichment usage and recorded behaviours.

Enrichment use/Behaviour	Definition/description
State behaviours	
Perch usage/Perching	Number of hens standing or resting on the wooden perch
Scratch mat	Number of birds standing or resting on the mat
Nest box/Nesting	Number of birds having their whole body or a part of its body in the nest box
Event behaviours	
Preening	Number of hens that preens its wings and feathers.
Pecking behaviour	Number of hens that gently or repeatedly pecks at the pecking block
Injurious pecking	Hen pecks at the feather or anterior of another bird.

4.3 Results

The results of the fixed effects of BE supplementation on the evaluated parameters is highlighted below, first the overall effects and then the tier effects.

4.3.1 Serum corticosterone, enrichment use, and behaviour

The BE supplementation resulted in a reduced (*P*=0.02) serum corticosterone (**SC**) concentration (18.00% lower) compared to the control over time, irrespective of the periods (Figure 4.1). A significant interaction (*P*=0.04) between bird age and BE supplementation was observed for perch use by hens in the afternoon in period 3 (wks 46 to 48). As shown in the period 3, the BE supplemented hens used the perches 2.06% more in the afternoon than in the control groups. However, no significance was observed between the treatments in the morning (Figures 4.2, Figure 4.3).

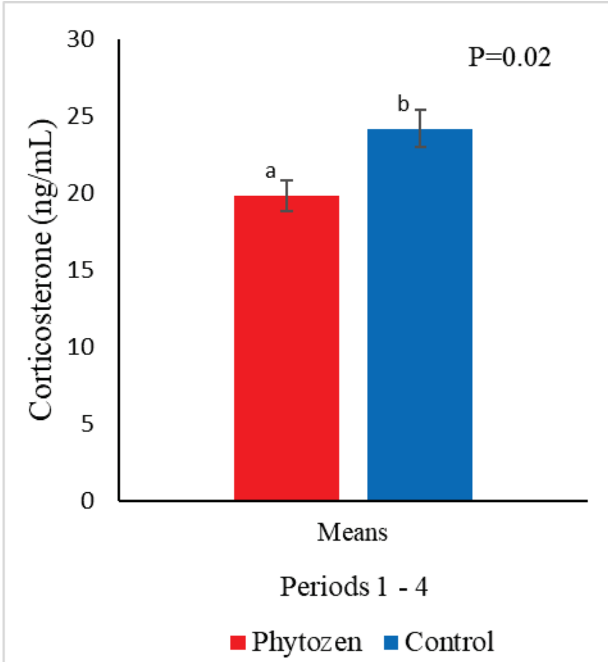

Figure 4.1. Mean serum corticosterone responses of Lohmann Lite hens housed in furnished cages to in-water BE supplementation measured at wks 40, 44, 48, and 52. Data points with different letters (a, b) are significantly different (***P*** < 0.05).

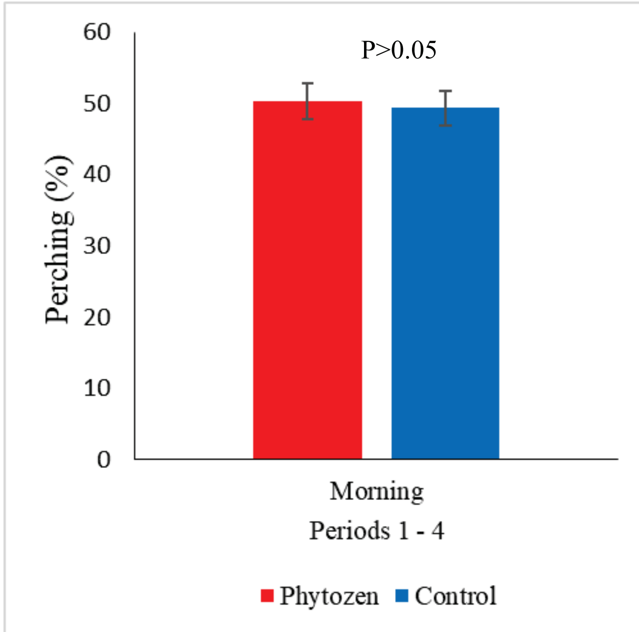

Figure 4.2. Percentage perch usage (mean ± √MSE) by Lohmann Lite hens, morning (am) given the in-water BE supplementation. Data points with different letters (a, b) are significantly different ($P < 0.05$).

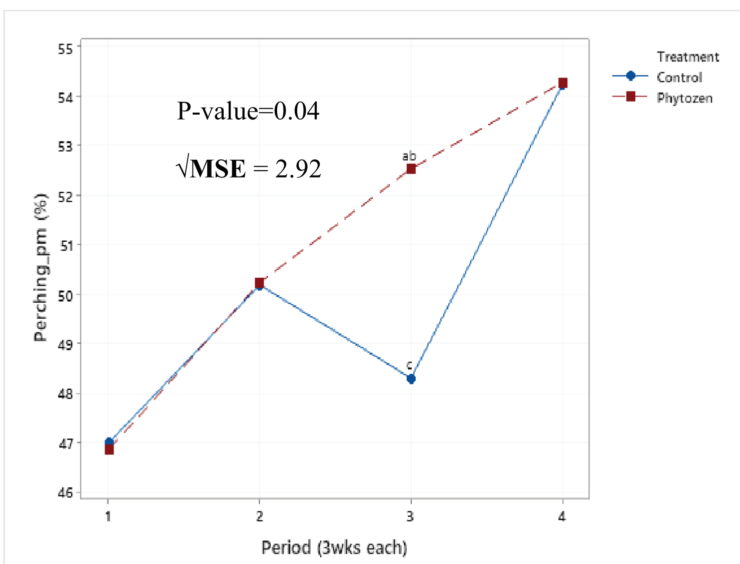

Figure 4.3. Percentage perch usage (mean ± √MSE) by Lohmann Lite hens, afternoon (pm), given the In-water BE supplementation. Data points with different letters (a, b) are significantly different ($P < 0.05$).

Similarly, the BE supplementation improved (P<0.01) scratch mat use in the afternoon by 6.44% compared to the control (Figure 4.3) throughout the trial. The BE increased (P<0.01) nest box use in the morning and afternoon (11.37% and 15.10%, respectively) compared to the control groups, irrespective of the period (Figure 4.4).

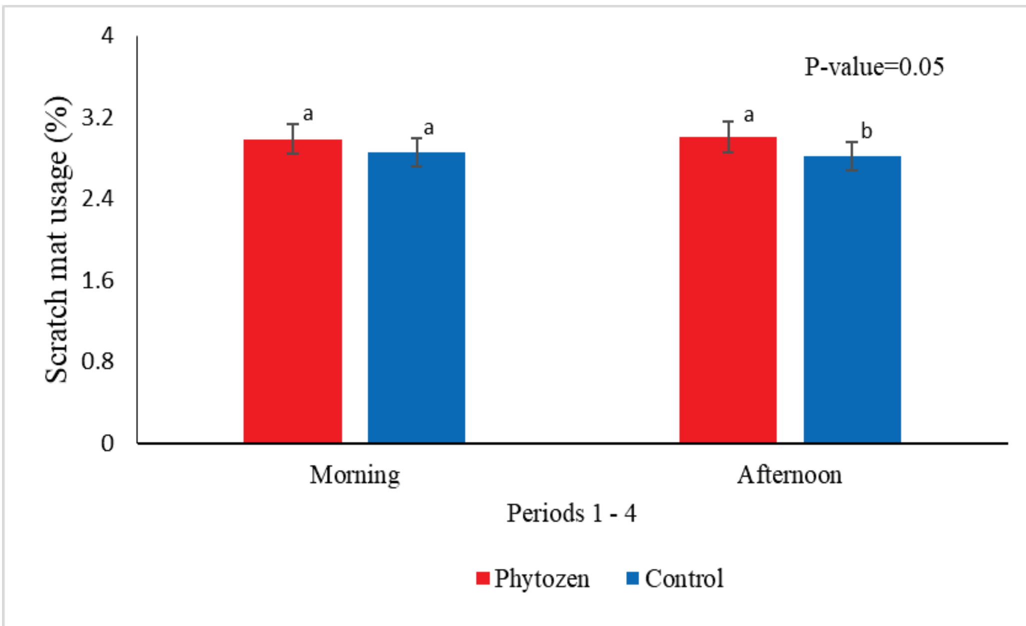

Figure 4.4. Percentage scratch mat usage (mean ± MSE) by Lohmann Lite hens, morning (am) and afternoon (pm), given the in-water BE supplementation. Data points with different letters (a, b) are significantly different (P < 0.05).

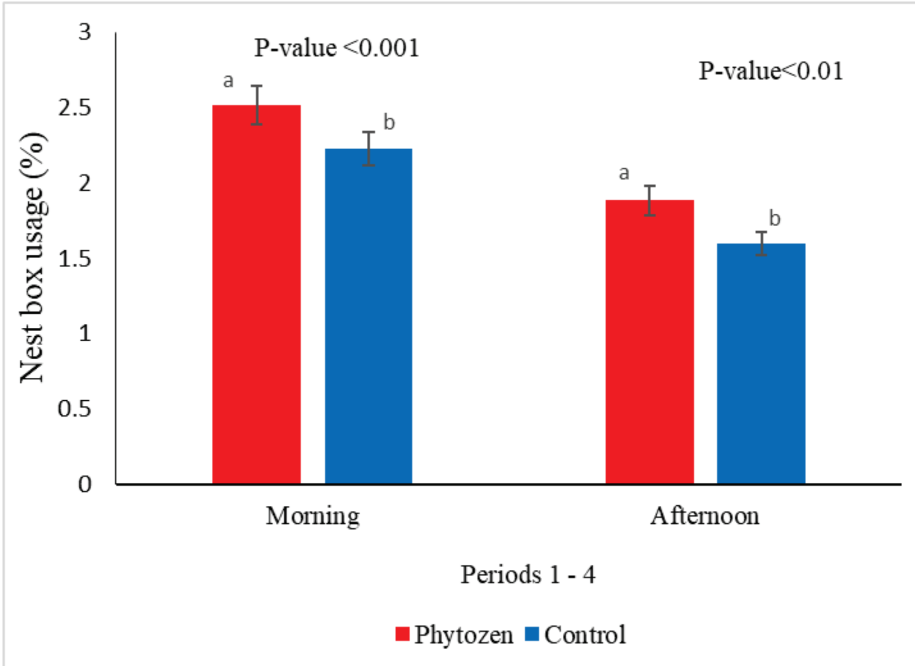

Figure 4.5. Percentage nest box usage (mean ± MSE) by Lohmann Lite hens, morning (am) and afternoon (pm), given the in-water BE supplementation. Data points with different letters (a, b) are significantly different ($P < 0.05$).

However, pecking block usage, pecking behaviour, preening, and injurious pecking were not affected (P>0.05) by BE supplementation, irrespective of the time of day and period (Figures 4.5 – 4.8). The injurious pecking behaviour data presented was not normally distributed due to the rarity of its occurrence from the scan samples. Table 4.4 summarizes the effects of phytozen on behaviour, enrichment usage, and serum corticosterone levels.

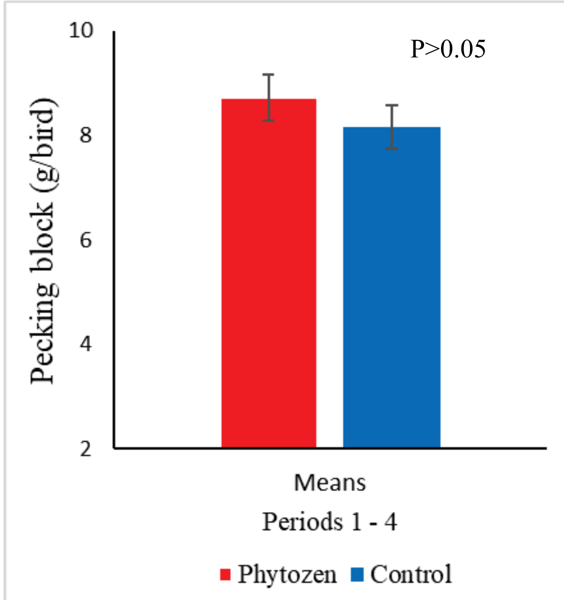

Figure 4.6. Pecking block use in g/bird (mean ± MSE) by Lohmann Lite hens given the in-water BE supplementation.

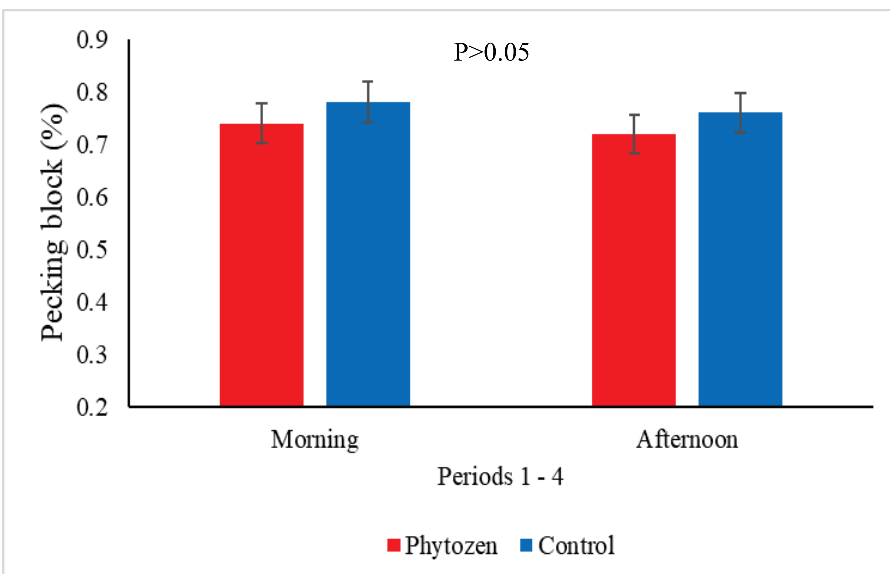

Figure 4.7. Percentage pecking behaviour (mean ± MSE) of Lohmann Lite hens, morning (am) and afternoon (pm), given the in-water BE supplementation.

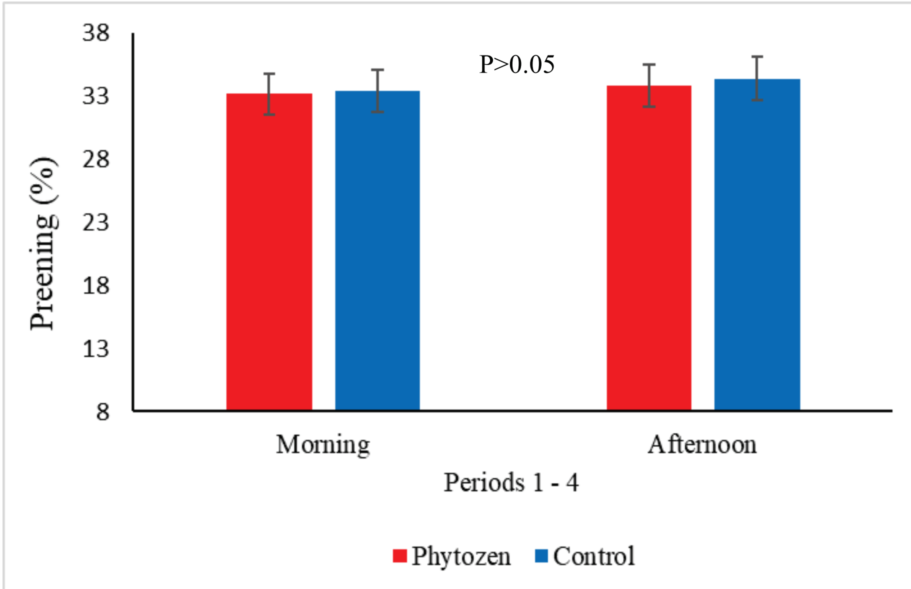

Figure 4.8. Percentage preening behaviour (mean ± MSE) of Lohmann Lite hens, morning (am) and afternoon (pm), given the in-water BE supplementation.

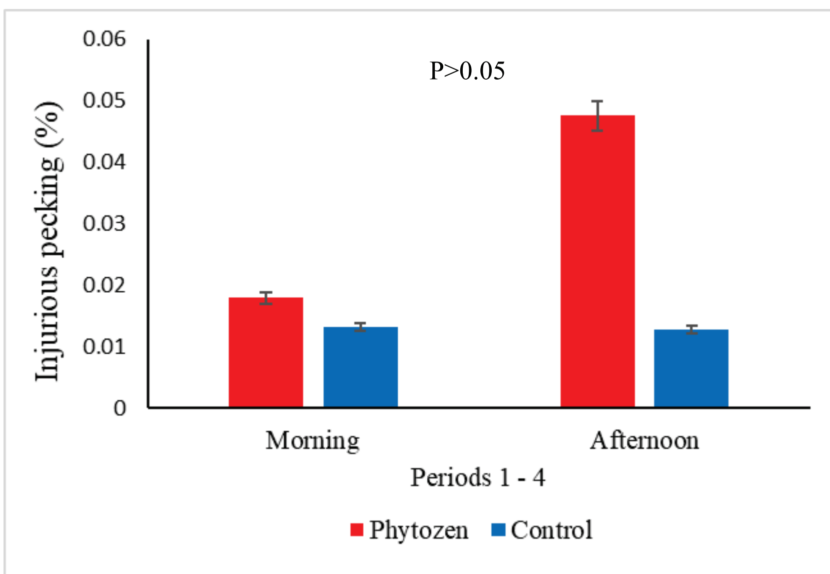

Figure 4.9. Percentage injurious pecking behaviour (mean ± MSE) of Lohmann Lite hens housed in furnished cages in response to the treatments.

4.3.2 Tier effect

Significant tier-treatment interaction (*P*<0.01) was on serum corticosterone levels. The **SC** of the bottom tier hens was lowest overall (17.44%) compared to the top tier hens. However, **SC** was lowest during BE supplementation to the top-tier hens and higher during in-water supplementation to the bottom-tier hens. Similarly, tier-treatment interactions (*P*<0.05) were also found for perches, scratch mats, and pecking block use (Table 4.3). Table 4 showed that the BE supplemented hens in the bottom-tier used perches more in the morning and afternoon, the nest box was used more by the top-tier hens in the morning and bottom-tier hens in the afternoon, and scratch mats in the bottom-tier were used more in the afternoon in the BE supplemented hens periods compared to the control groups. Furthermore, a tier versus treatment interaction (*P*=0.007) was observed for pecking block usage.

Table 4.2. Tier effect and Tier×treatment interaction of a botanical extract on enrichment usage, behaviour and stress response of Lohmann Lite hens housed in a furnished cage system

Parameter	Treatment					Tier			P-value		
	Tier1 Phytozen	Tier 2 Phytozen	Tier 1 Control	Tier 2 Control	SEM	Tier 1 Overall	Tier 2 Overall	SEM	Treatment	Tier	Trt*Tier Interaction
Enrichment/State behaviour (%)											
Perches (am)	50.26^{a}	50.71^{a}	50.68^{a}	47.82^{b}	0.606	50.47	49.26	0.634	0.066	0.059	0.011
Perches (pm)	49.71^{a}	52.27^{a}	52.19	47.64^{b}	0.621	50.95	49.95	0.747	0.039	0.186	<0.001
Nest box (am)	2.59^{a}	2.45^{a}	2.20^{b}	2.26^{a}	0.069	2.40	2.35	0.073	<0.001	0.545	0.177
Nest box (pm)	1.81^{a}	1.95^{a}	1.68^{a}	1.52^{b}	0.06	1.88^{a}	1.60^{b}	0.080	0.003	0.924	0.084
Scratch mat (am)	2.88	3.09	2.86	2.85	0.085	2.87	2.97	0.057	0.229	0.094	0.053
Scratch mat (pm)	2.92^{a}	3.09^{a}	2.85^{a}	2.78^{b}	0.074	2.89	2.94	0.055	0.048	0.053	0.053
Enrichment/Event behaviour (%)											
Preening (am)	33.23	33.07	34.61	32.20	0.43	33.92	32.63	0.626	0.567	0.043	0.077
Preening (pm)	34.61	33.15	36.24	32.57	0.643	35.43^{a}	32.86^{b}	0.643	0.436	<.001	0.090
Pecking object (am)	0.77	0.71	0.78	0.77	0.043	0.77	0.74	0.074	0.596	0.465	0.531
Pecking object (pm)	0.75	0.70	0.81	0.72	0.043	0.78	0.71	0.052	0.483	0.103	0.668
Stress response											
Corticosterone (ng/mL)	15.10^{c}	24.16^{b}	33.06^{a}	15.60^{c}	1.81	24.08^{a}	19.88^{b}	1.93	0.016	0.031	<0.001
Pecking block (g/bird)	7.16	10.31	11.47	5.08	1.819	9.31	7.70	1.287	0.810	0.354	0.007

Note: SEM, standard error of the mean. Means along the same row with different superscript are significantly different at P < 0.05.
Tier 1 (Top cages); the overall effect of the BE extract on the parameters taken on birds from the top cages.
Tier 2 (Bottom cages); the overall effect of the BE extract on the parameters taken on birds from the bottom cage; a, b, P<0.05
Percentage (%); all state and event enrichment/behaviours are presented as a percentage of total observations

Table 4.4. Summary of the Effects of in-water Phytozen supplementation on production behaviour, enrichment use and stress response from 38-52 weeks age in laying hens

	Treatment Means		P-value			SEM
	Control	Phytozen	Trt	Period	Trt × Period	
Enrichment/State behaviour (%)						
Perches (am)	46.80	47.79	0.16	<0.01	0.15	0.62
Perches (pm)	49.92 b	50.97 a	0.03	<0.01	0.04	0.44
Nest box (am)	2.23	2.52	<0.01	<0.01	0.14	0.06
Nest box (pm)	1.59 b	1.88 a	<0.01	0.17	0.30	0.08
Scratch mat (am)	2.85 b	2.99 a	0.23	<0.01	0.41	0.11
Scratch mat (pm)	2.82 b	3.01 a	0.05	0.01	0.61	0.09
Enrichment/Event behaviour						
Preening (am)	33.45	33.17	0.53	<0.01	0.09	0.44
Preening (pm)	34.38	33.85	0.36	0.02	0.06	0.30
Pecking block (am)	4.61	4.62	0.99	<0.01	0.12	0.44
Pecking block (pm)	4.52	4.48	0.85	0.01	0.71	0.25
Stress response						
Serum corticosterone (ng/mL)	24.17 a	19.82 b	0.02	<0.01	0.13	1.67
Pecking block (g/bird)	8.14	8.71	0.76	<0.01	0.15	1.85

Note: Percentage (%); all state and event enrichment/behaviours are presented as a percentage of total

4.4 Discussion

Previous studies across many animal species have shown the compounds in Phytozen® to enhance mental-well being, increased activity, reduce fear and stress response, as well as promote calm behaviours (Brown et al., 2017; Julien et al., 2021; Perveen et al., 2009; Noirot, 2017; Raza et al., 2006). This is consistent with the results of stress response and enrichment use obtained in this study. The BE supplementation affected the use of enrichment and behaviour occurrences at a given time. This study found that in-water supplementation of botanical extract significantly lowered circulating stress hormone, enhancing the overall physiological welfare of Lohmann Lite hens. Therefore, this section discusses how the study's significant results compare to other scientific reports and possible reasons for the observed tier-treatment interactions.

4.4.1 Serum corticosterone

In avian species, the level of circulating corticosterone is an important indicator of stress response. Neuroendocrine, behavioural, and physiological responses in birds have been investigated extensively. Under normal conditions, poultry birds produce some corticosterone, but changes in the circulating concentration could be due to various stressors, such as handling (Radke et al., 1985; Silverin, 1998), stocking density and social stress (Mashaly et al., 1984; Shini, 2003), fear (Fraisse and Cockrem, 2007; Labar and Ledoux, 2001), vitamin metabolism (Mahmoud et al., 2014; Saiz del Barrio et al., 2020; Satterlee et al., 1993), presence of environmental enrichments (Shi et al., 2019), housing type changes in housing environments (Abdel-Hamid et al., 2020; Kang et al., 2016; Koelkebeck and Cain, 1984) and so on.

Phytogenic additives are well-known for their antioxidant properties. In this study, the botanical extract blend containing rosemary oil, turmeric, magnesium sulphate, sweet orange, and ascorbic acid consistently lowered the circulating levels of serum corticosterone assessed four times during the trial. The exciting result of stress reduction could be attributed to the antioxidant properties of the BE extracts, as indicated in the literature. According to Julien et al. (2021), the BE blend had a favourable effect on stress mitigation, characterized by faster and consistent recovery from fear and lower bodily stress induced by a sudden increase in stocking density. Turmeric extracts and rosemary oil have also been shown to inhibit prostaglandin E2 in response to oxidative stress (Menghini et al., 2010). The result of stress hormone reduction in this study is also consistent with earlier reports on furnished housing compared with other housing systems (Krivankova et al., 2020; Matur et al., 2015; Pohle and Cheng, 2009). Similarly, Shi et al. (2019) found that layer breeders housed in colony cages with nest boxes secreted lower plasma corticosterone than the control group. This information highlights the welfare benefit of stress reduction of phytogenic additive blend on egg-laying chicken in an enriched housing system.

Blas et al. (2005) and Fraisse and Cockrem (2007) were the first to study tier differences in corticosterone levels to stress-related responses or unpredictable events in their surroundings. Blas et al. (2005) found acute stress hormone levels in older nestlings. In contrast, Fraisse and Cockrem (2007) observed no significant tier differences in plasma corticosterone concentrations between White Leghorn and Brown Hyline hens. The tier disparities identified in this study can be partially attributed to the influence of the BE, as evidenced by the lowest concentrations reported during the BE supplemented period to the top tiers (Table 4.3). Previous studies have found that broiler chickens exposed to continuous bright light had higher plasma corticosterone levels than subjects exposed to limited light; however, this effect could be transient and depend on different light intensities (Kang et al., 2020; Lauber et al., 1987). This finding implies that the BE blend has anxiolytic properties, as our earlier case report predicted on performance and egg quality, where the BE supplementation regulated the water/feed ratio of top-tier hens with more exposure to light. Other unknown factors could account for the high corticosterone levels in the bottom tiers during the BE supplementation. However, tier, strain, and age differences should be considered when analyzing the physiological response of hens to botanical extracts in future studies.

4.4.2 Enrichment use

There is no literature on the capacity of phytogenic additives to promote the use environmental enrichment items such as perches, scratch mats, nest boxes, and pecking objects in laying hens. The botanical extract blend in the current study demonstrates some evidence of improved usage of perches and perching behaviour, scratch mats, and nest box. The effect of the botanical extract blend on Lohmann Lite hens' use of nest boxes was significant regardless of the time of day. In contrast, significant outcomes for perches and scratch mats were only observed in the afternoon. Because increasing enrichment use can be equivalent to enhanced expression of natural behaviour, for the sake of this discussion, the terms enrichment use and behaviour may be interchanged.

4.4.2.1 Perch usage

Perching is an important natural behaviour that improves hen well-being. Among notable results of perch use in broiler chickens and laying hens are, increased activity and bone strength (Hester, 2014; Kaukonen et al., 2017), an opportunity for vulnerable hens to flee their aggressive mates (Appleby, 2003; Appleby et al., 1992), and access to roosting (Hemsworth and Edwards, 2020; Hongchao et al., 2013). Naturally, birds' preference for perching increases with increasing time of day, particularly at night (Campbell et al., 2016; Olsson and Keeling, 2000). Similarly, factors such as the strain (Faure and Jones, 1982), age (Levan et al., 2000), stocking density, and type of perch material could impact its utilization (Faure and Jones, 1982; Hester et al., 2013; Hongchao et al., 2013). Although Lohmann Lite hens in the BE treatment groups used the perches more in the afternoon than in the morning at certain number of weeks (46 – 48wks), it is not clear the mechanism by which this happened. We can consider this an eye-opening discovery, warranting further investigation. Consistent with the behaviour of their wild predecessor (the red junglefowl), a higher percentage of birds roost at night (Abrahamsson et al., 1996; Duncan et al., 1992). According to Liu and Xin (2017), the majority of Lohmann Lite hens' perching activities occurred during the dusk-like period. The current study's finding suggests that BE supplementation enhanced increased use of perches during the day, but this cannot be directly linked to higher activities as other factors could be at play. To broaden this finding, other variables, including interactions between strain, perch type, cage size, and aggressive pecking activities must be correlated with the time of day.

4.4.2.2 Nest box usage

The use of nest boxes in furnished cages for laying hens is essential for improving good experiences and the rate of saleable eggs. However, factors such as the substrate type and location (Struelens et al., 2005) influence the attraction or acceptance of a nesting site. Artificial turf (Campbell et al., 2017; Struelens et al., 2005) and straw-bedded floor (Campbell et al., 2017) are common substrates; however, the latter is not sustainable because of the need for continual replenishment. Social factors such as the presence of dominant hens, delayed oviposition, or preference for other non-nesting sites could all influence hens' usage of nest boxes (Oliveira et al., 2016; Reynard and Savory, 1999; Rietveld-Piepers et al., 1985). The artificial turf utilized for the scratch mats was used to line the nest box in the furnished cage used for this research.

All eggs were laid in the nest box in this study. It is also possible that the cage's size made all hens lay their eggs in the private nesting site. According to Hunniford et al. (2014), hens aggressively competed for the available nesting site in a moderately sized furnished cage, whereas they chose other nesting sites in bigger furnished cages. It is important to note that the improvement in nest box use was enhanced by the botanical extract supplementation irrespective of the period or the time of the day compared to the control periods. This could imply that the BE made the hens more relaxed in the nest boxes, or possibly because the substrate in the nest box was the same as with the scratch mats. On the other hand, we speculate that there is a possible relationship between nest box use and reduction in serum corticosterone levels. Shi et al. (2019) reported that breeder hens with access to nest boxes had lower corticosterone levels compared to the control. This explanation does not entirely fit into this report since all birds had access to nest box, however, stress in an ongoing activities in every housing type and the mitigating effort of the BE blend in reducing serum corticosterone could suggests the improvements in nest box visits. In addition to the nest box use, our results showed a higher laying rate in the afternoon among the hens that received the botanical extract blend (in period 1) than in the control group (see supplementary figures 2a and b).

4.4.2.3 Scratch mat usage

The presence or absence of exploratory behaviour has been used to evaluate the welfare of confined birds. Scratch mats could stimulate exploratory behaviour, such as foraging and scratching, compared to barren cages (Lay et al., 2011; Pokharel et al., 2018). In furnished cages, the wire floor precludes this natural behaviour. This natural behaviour can be mimicked by laying scratch mats on a wire floor and sprinkling a small amount of feed on the mats. (Sandilands et al., 2021). The significant effect of the BE on scratch mat usage suggests that the extract blend has the capacity to stimulate happy emotions and natural behaviour (exploratory) in the hens by increasing the frequency of visits or use of scratch mats. The anxiolytic effects of rosemary oil and sweet orange have been reported in male wistar rats leading to increased exploration and restoration of neuronal cells (Faturi, 2010; Rahbardar et al., 2020).

The result of the scratch mat used in this study could further suggest that its use may have been influenced by the size, accounting for the few numbers of birds found using it at each time scan. Compared to alternative housing systems, such as the aviary (where wood shavings are used as litter materials), the size of a scratch mat placed in the enriched cage is a function of the cage size. Similar to the results obtained by Sandilands et al. (2021), Li et al. (2016) also reported that scratching behaviour was higher in the moderately bigger furnished cages (medium furnished cage type III) compared to other smaller types of furnished cages and conventional cages. Thus, the size of scratch mats may be an important element influencing birds' propensity to utilize them more frequently in enriched cages. Hence, a single hen may occupy the mat for an extended period, preventing access to other birds. The current findings are consistent with previous research by Rieke et al. (2021) and Channing et al. (2001), in which laying hens were shown to spend more time performing active behaviours in the afternoon.

Pecking block usage and preening behaviour of Lohmann Lite hens were not different between the treatments. Bouts of injurious pecking behaviour were rare, and its occurrence is represented in Figure 4.9. Increased use of enrichment objects, including scratch mats, indicates increased opportunity for expression of exploratory behaviour by hens in the botanical extract supplemented groups. This study is the first attempt at examining the potential of the combinative use of phenolic-rich plant extracts in improving the environmental enrichment use of laying hen in furnished housing systems.

4.5 Conclusions and recommendations

This study concludes that this proprietary blend of botanical extracts containing rosemary oil, turmeric, sweet orange, ascorbic acid, and magnesium sulphate in the drinking water of Lohmann Lite hens showed some evidence of improved usage of perches, nest boxes, scratch mats, and reduced overall levels of serum corticosterone. A combination of these positive experiences, including reduced stress response and increased enrichment usage, contributes to improved welfare of Lohmann Lite hens in furnished cages. Results from the tier versus treatment interactions also provoke curiosity about the possible relationship between the blend of botanical extracts and light intensity.

The study aimed to replicate commercial laying hen housing conditions. However, there were cases of image occlusion which was due to the group size used in the study. For ease of sampling, fewer birds are proposed for future studies. It is also recommended that the strain-strain interaction of laying hens with different housing types should be investigated for the Phytozen®Liquid product range. In addition, further studies on Phytozen®Liquid could involve a mild environmental stress challenge on furnished-caged-housed laying hens. It is also important that potential limitations of this study—including the number of replicates be taken into consideration in order to aid proper interpretation of results presented.

CHAPTER 5: Conclusions

5.1 Conclusions

The combinative effect of the phytogenic additive blend (Phytozen®Liquid) showed some significant evidence of improved production and welfare of Lohmann lite hens housed in a furnished-cage system. The botanical extract blend improved feed intake at 40wks (period 1), egg weight at 44wks (period 2), and enhanced albumen quality throughout the trial. Furthermore, the extract blend also showed improved use of enrichment objects (scratch mats, nest boxes, and perches) while decreasing circulating stress hormone throughout the trial period. To avoid overstating the extract blend's effects, it is apparent that some of the significant effects reported in this study varied with the age of the birds. Future research should attempt to administer Phytozen at a much earlier stage of their laying cycle. This study also inferred that proximity to light could influence the behaviour and performance of laying hens, regardless of the treatment. In conclusion, Phytozen®Liquid has the potential to improve the overall welfare of laying hens in furnished cages.

The result of this study highlights the potential economic benefits of this proprietary blend of botanical extract on the welfare metrics of laying hens in furnished cages. By implication, the results of this study will a) enhance the increasing adoption and awareness of the economic benefits of furnished cages in Canada; b) contribute towards the 2036 goal of phasing conventional cage use out of Canada; c) promote the acceptance of safe and healthy egg and egg products made from commercially available plant extracts, by the public.

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APPENDIX

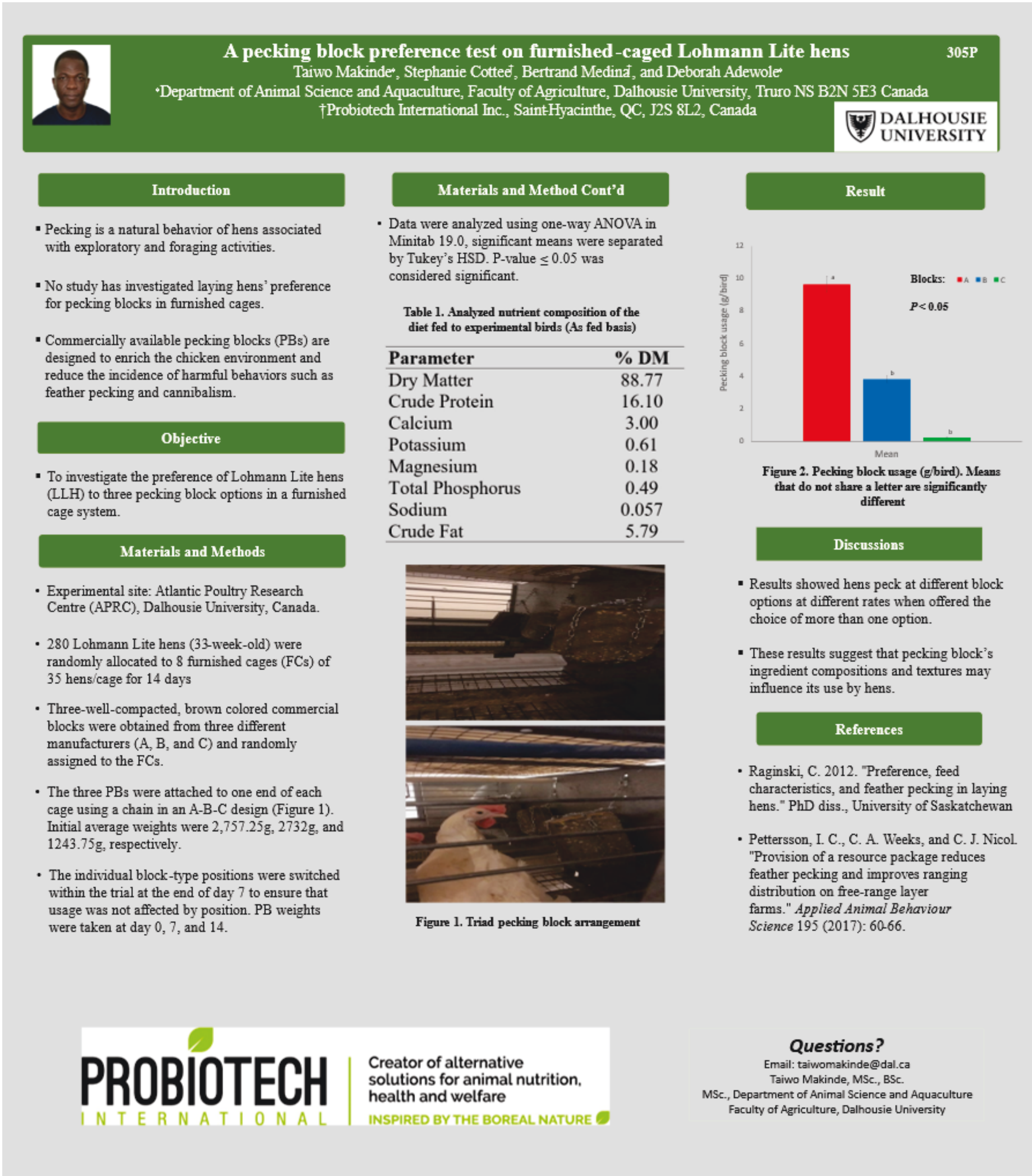
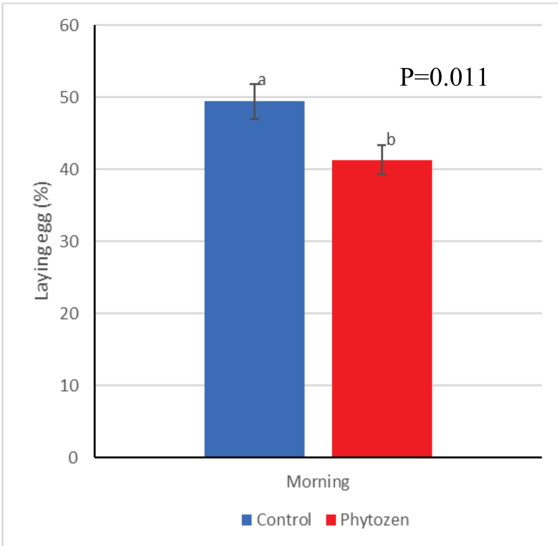
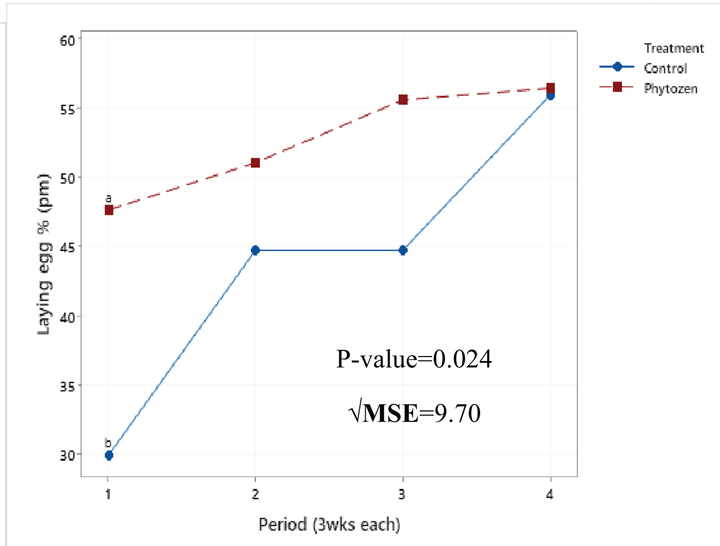
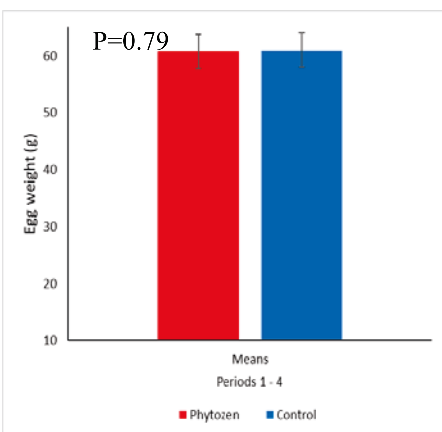


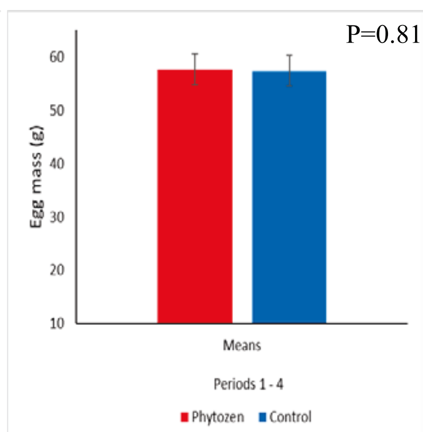
Figure 1. A pecking block preference test on furnished-caged Lohmann Lite hens. A poster presented at Poultry Science Association Annual Meeting, 2022.





Figures 2a, b. Laying egg percentage (mean ± √MSE) by Lohmann Lite hens, morning (am) and afternoon (pm), given the In-water BE supplementation. Data points with different letters (a, b) are significantly different ($P < 0.05$).





Figures 3a, b. Effect of BE on eggshell breaking strength of Lohmann Lite hens. Values are expressed as means ± SE. Data represent overall differences between treatment groups