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UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
CAMPUS DE BOTUCATU

USO DE LIGNINA PURIFICADA NA DIETA DE POEDEIRAS COMERCIAIS CRIADAS EM DIFERENTES SISTEMAS E AMBIENTE TERMO ESTRESSANTE

ANA BEATRIZ SANTOS DE OLIVEIRA

Tese apresentada ao Programa de Pós-graduação em Zootecnia como parte das exigências para obtenção do título de Doutora em Zootecnia.

BOTUCATU – SP
Outubro - 2023

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Á minha mãe, Maria de Lurdes, por ser tudo na minha vida.

Ao meu pai, Paulo Roberto, por todos os momentos de carinho, apoio e compreensão.

Aos meus avós, Luzia e José, por todo amor.

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EPÍGRAFE

*“**Viver é partir, voltar e (re)partir***
Morte é quando a tragédia vira um costume
Partir, voltar e (re)partir
Pra diferença da qual ninguém 'tá imune
Viver é partir, voltar e (re)partir
Mas ouça de alguém que nasceu num tapume
Partir, voltar e (re)partir
É só na escuridão que se percebe os vagalumes”.
(Emicida)

RESUMO GERAL

Três experimentos foram conduzidos para avaliar os efeitos dos níveis de lignina purificada (LP) em galinhas poedeiras. O primeiro experimento (**Exp. 1**) teve como foco o desempenho, saúde intestinal, qualidade e tempo de prateleira dos ovos de galinhas criadas em sistema convencional. No segundo experimento (**Exp. 2**), foram analisadas a produção, qualidade de ovos, morfologia e saúde intestinal de poedeiras em sistema *cage-free*. E no terceiro experimento (**Exp. 3**), o objetivo foi avaliar a produtividade, qualidade de ovos e morfologia intestinal de poedeiras em sistema *cage-free* sob elevadas temperaturas. Foram utilizadas 240 galinhas da linhagem Hy-Line W-36 com idade entre 31 e 43 semanas no **Exp. 1** e 300 galinhas, com idades entre 51 e 63 semanas no **Exp. 2** e 63 a 66 semanas no **Exp. 3**. Os animais foram submetidos a cinco tratamentos no **Exp. 1**, com seis repetições contendo oito aves por gaiola. No **Exp. 2 e 3**, foram utilizados dois tratamentos, com três repetições e 50 aves por baia. No **Exp. 1** os tratamentos consistiram em cinco dietas: uma dieta controle (DC) e quatro dietas testes suplementadas com LP nos níveis de 0,25%, 0,5%, 0,75% e 1%. Nos **Exp. 2 e 3** foram utilizados dois tratamentos sendo uma DC, e adotou-se o nível de destaque do **Exp. 1**, que foi o nível de 1% de suplementação de LP. **Exp.1:** Os tratamentos apresentaram $P<0,05$ em vários parâmetros ao longo dos ciclos experimentais. Destacam-se as diferenças na produção, peso, massa dos ovos e consumo de ração em diferentes ciclos. A concentração de malondialdeído (MDA) variou significativamente, indicando a influência positiva da adição de LP na redução da oxidação lipídica na gema do ovo. Além disso, a qualidade dos ovos, medida pela espessura da casca e gravidade específica, também foi afetada pelos tratamentos. **Exp. 2:** Nos resultados relacionados aos parâmetros produtivos e de desempenho observou-se que os tratamentos exibiram $P<0,05$ ao longo dos ciclos experimentais, impactando a produção, massa e peso dos ovos, bem como a proporção de ovos postos dentro e fora dos ninhos. Em relação aos parâmetros de qualidade dos ovos, os tratamentos também apresentaram $P<0,05$ ao longo dos ciclos experimentais, particularmente quanto à gravidade específica e espessura da casca. Notavelmente, a concentração de MDA demonstrou $P<0,05$ em todas as semanas experimentais. Nas análises relacionadas à morfologia e saúde intestinal, observaram-se $P<0,05$ no peso do baço e do jejuno, além do tamanho dos vilos presentes no jejuno. Entretanto, não se constatou efeito significativo nos níveis de ácidos graxos de cadeia curta (AGCC) em virtude da suplementação de LP. **Exp. 3:** Nos parâmetros produtivos e de desempenho, os tratamentos mostraram $P<0,05$. A suplementação de LP teve impactos negativos na conversão alimentar e na proporção de ovos postos dentro e fora dos ninhos. Quanto aos parâmetros de qualidade dos ovos, houve $P<0,05$, a suplementação de LP aumentou a porcentagem de casca, mas reduziu a porcentagem de gema. A concentração de MDA apresentou $P<0,05$ durante um estresse agudo de calor. No entanto, não foi observado efeito ($P>0,05$) quando a exposição a altas temperaturas foi prolongada. Nas análises referentes à morfologia e saúde intestinal, notaram-se $P<0,05$ no comprimento dos vilos do duodeno. Entretanto, não se constatou efeito significativo nos níveis dos AGCC e nas análises dos órgãos internos devido a suplementação de LP. Conclui-se que, em um sistema criação convencional, a dosagem de 1% de LP foi segura e não afetou o desempenho das poedeiras. No entanto, no sistema *cage-free*, a inclusão de 1% de LP na dieta de galinhas resultou em desempenho inferior quando comparado ao DC. Contudo, sob condições de elevadas temperaturas ambientais, a suplementação de 1% de LP na dieta de galinhas em sistema *cage-free*, não afetou o desempenho das poedeiras. Porém, em ambos os sistemas de criação e condições ambientais foi observada melhora na qualidade dos ovos, devido ao seu efeito antioxidante, sem impacto na morfologia e histologia intestinal das galinhas.

Palavras-chave: antioxidante, sistemas de criação, qualidade de ovo, tempo de prateleira, ácidos graxos de cadeia curta

ABSTRACT

Three experiments were carried out to evaluate the effects of purified lignin (PL) levels in laying hens. The first experiment (**Exp. 1**) focused on the performance, intestinal morphology, quality, and shelf life of eggs from hens raised in a conventional system. In the second experiment (**Exp. 2**), production, egg quality, intestinal morphology, and histology of laying hens in a *cage-free* system were analyzed. The third experiment (**Exp. 3**), the objective was to evaluate productivity, egg quality and intestinal morphology and histology of layers in a *cage-free* system under high temperatures. 240 Hy-Line W-36 hens aged between 31 and 43 weeks were used in **Exp. 1** and 300 hens aged between 51 and 63 weeks in **Exp. 2** and 63 to 66 weeks in **Exp. 3**. The hens were subjected to five treatments in **Exp. 1**, with six replications containing eight birds per cage. In **Exp. 2 and 3**, two treatments were used, with three replications and 50 birds per pen. In **Exp. 1**, treatments consisted of five diets: a control diet (CD) and four test diets supplemented with PL at levels of 0.25%, 0.5%, 0.75% and 1%. In **Exp. 2 and 3**, two treatments were used, one DC, and the highlighted level of Exp. 1 was adopted, which was the 1% level of PL supplementation. **Exp.1:** The treatments showed $P<0.05$ in various parameters throughout the experimental cycles. Differences in production, weight, egg mass and feed intake in different cycles are highlighted. The concentration of malondialdehyde (MDA) varied significantly, indicating the positive influence of PL addition on reducing lipid oxidation in egg yolk. Furthermore, egg quality, measured by shell thickness and specific gravity, was also affected by treatments. **Exp. 2:** In the results related to production and performance parameters, it was observed that the treatments exhibited $P<0.05$ throughout the experimental cycles, impacting the production, mass, and weight of eggs, as well as the proportion of eggs laid inside and outside the nests. In relation to egg quality parameters, treatments also showed $P<0.05$ throughout the experimental cycles, particularly regarding specific gravity and shell thickness. Notably, MDA concentration demonstrated $P<0.05$ in all experimental weeks. In analyzes related to intestinal morphology and histology, $P<0.05$ were observed in the weight of the spleen and jejunum, in addition to the size of the villi present in the jejunum. However, there was no effect on the levels of short-chain fatty acids (SCFA) due to PL supplementation. **Exp. 3:** In productive and performance parameters, the treatments showed differences ($P<0.05$). PL supplementation had negative impacts on feed conversion ration and the proportion of eggs laid inside and outside the nests. Regarding egg quality parameters, there were variations ($P<0.05$). PL supplementation increased the percentage of shell but reduced the percentage of yolk. MDA concentration showed differences ($P<0.05$) during acute heat stress. Though no effect was observed ($P>0.05$) when exposure to high temperatures was prolonged. In the analyzes regarding morphology and histology intestinal, differences (P<0.05) were noted in the length of the Duodenum villi. However, no effect was found on SCFA levels and internal organ analyzes due to PL supplementation. In conclusion, in a conventional breeding system, the dosage of 1% PL was safe and did not affect the performance of the layers. Though, in the *cage-free* system, the inclusion of 1% PL in the hens diet resulted in lower performance when compared to DC. Nonetheless, under conditions of high environmental temperatures, supplementation of 1% PL in the diet of hens in a *cage-free* system did not affect the performance of the layers. Conversely, in both breeding systems and environmental conditions, an improvement in egg quality was observed, due to its antioxidant effect, without impact on the intestinal morphology and histology intestinal of the hens.

Keywords: antioxidant, breeding systems, egg quality, shelf life, short chain fatty acids

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

BW	Body Weight
BWG	Body Weight Gain
cm	Centimeters
EM	Egg mass
EP	Egg Production
EW	Egg weight
FCR	Feed Conversion Ratio
FI	Feed Intake
FMVZ	Faculdade de Medicina Veterinária e Zootecnia
g	Grams
IML	Interactive Matrix Language
kfg	Kilogram Force
kg	Kilos
m	Meters
MDA	Malondialdehyde
ml	Milliliters
mm	Millimeters
Na_2S	Sodium Sulfide
NaOH	Sodium Hydroxide
ng	Nanogram
PL	Purified Lignin
ROS	Reactive Oxygen Species
SCFA	Short Chain Fatty Acids
SP	São Paulo
t	Ton
UNESP	Universidade Estadual Paulista
µm	Microgram

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CAPÍTULO 1

INTRODUCTION

Poultry farming is an agricultural subsector that presents itself as one of the most developed activities in the world, with an active contribution to food and nutritional security, providing energy, protein, and essential micronutrients for the human population. Poultry meat and eggs are among the highest consumed animal protein sources globally (EL-HACK et al., 2020). Brazil has had significant climb in the *per capita* consumption of eggs in the last 20 years, as well as its production. A total of 52 billion eggs were produced in Brazil in 2022 (ABPA 2023).

Eggs are considered one of the richest foods nutrition wise for human consumption, as it is a source of essential proteins, vitamins, minerals, and fatty acids for people's daily diet (RÊGO et al., 2012). However, several factors influence the quality such nutritional treasure, making it impossible to take advantage of its full potential. Among these, we might list bird physiology, breeding system, number of hens per cage, frequency of collection, age of birds, nutrition, management conditions, health status, temperature and humidity, genetics, and birds' management (SACCOMANI, 2015).

The supplementation of poultry diets with natural products containing bioactive components has shown promising results in terms of improving the birds' physiology and consequently their productivity. Studies have shown that purified lignin has effects on gut microflora, animal performance and their ability to inhibit the growth of pathogenic enteric bacteria (RICKE et al., 1982; NELSON et al., 1994; GUARRERA, 1999; BAURHOO et al., 2007a; BAURHOO et al., 2008).

As a polyphenolic compound, purified lignin exhibits antioxidant properties by neutralizing reactive oxygen species and protecting cells against oxidative damage (FERNANDES et al., 2015; MARCHIORI et al., 2019; LIU et al., 2020). In addition, purified lignin also contains anti-inflammatory properties, reducing the inflammatory response in a few conditions. These combined effects make purified lignin an optimistic option as a dietary additive, contributing to birds' health and welfare, as well as to optimizing productive performance (CATIGNANI; CARTER, 1982; BAURHOO et al., 2007a; OHISHI et al., 2016).

Heat stress causes severe physiological dysfunctions that can result in a decline in laying hen productivity (GOUS; MORRIS, 2005). High temperatures can be detrimental to laying hens, as it contributes to increased mortality, reduced number and quality of eggs and high formation of free radicals in the body and reproductive system (GOUS; MORRIS, 2005). The use of polyphenols may help laying hens by reducing the harmful effects produced during heat stress.

LITERATURE REVIEW

1. Egg Market

The egg market is a constantly growing sector driven by several factors, such as changing consumer preferences, demand for healthier food options and awareness of animal welfare practices (SANTOS, 2021a). Demand for eggs as a food has increased due to people's awareness of their health benefits, not to mention their culinary versatility and affordability compared to other protein sources (GODINHO JR et al., 2018; SANTOS et al., 2019; NASCIMENTO SILVA, 2020).

Additionally, the demand for eggs produced in sustainable farming systems and with animal welfare has increased significantly (CANDIDO, 2022). Consumers are increasingly concerned about the origin of their food and look for options that respect the environment and allow the animals to express natural behaviors (MAZZUCO, 2008; CARVALHO, 2019; REIS, 2022). As consumers become more aware of the origin of their food and seek more natural options, alternative farming systems, such as *cage-free* and *free-range*, are gaining ground in the market (COUNCIL, 2009; DE OLIVEIRA et al., 2019).

Cage-free and *free-range* system are more ethical and sustainable options, which is aligned with consumer preferences that value responsible food production (PARKER AND SCRINIS, 2014; DE OLIVEIRA et al., 2019). These systems promote hens' freedom, allowing them to have contact with their natural environment, exercise and express their natural behaviors, which contributes to their welfare (REIS, 2022).

Eggs from these systems are marketed as superior quality products, which can result in higher demand and price in relation to eggs from conventional systems. Few consumers are willing to pay the extra dollar for eggs produced in such premium systems (BRIDI et al., 2020; DE OLIVEIRA et al., 2019). Therefore, the search for solutions that contribute to improving egg production and, at the same time, reduce costs are being widely explored.

It is important to emphasize that the transition to alternative breeding systems may require investments and adjustments in properties' infrastructure, in addition to demanding more complex management (ARNO, 2022). Producers might have to consider aspects such as biosecurity, proper management, balanced and preventive feeding, to guarantee the production of safe and high-quality eggs (DE SÁ et al., 2023; ARNO, 2022).

The egg market is on the rise, driven by the demand for nutritious, versatile, and accessible foods (DE SOUZA CUNHA et al., 2019). Awareness of nutritional benefits and sustainable rearing systems drive the adoption of alternative rearing systems, impacting the supply, quality, and commercialization of eggs in the market (BRIDI et al, 2020; MAHMOUD et al., 2022). Producers' response to these demands reflects the constantly evolving dynamics of the egg production sector (ROCHA et al., 2008; DE SÁ et al., 2023).

2. Laying hens systems

2.1. Conventional system

The cage system came from the need to house each bird individually to allow individual recording of egg production and the disposal of unproductive hens. Afterwards, several birds were housed in a cage, this being the most common way of housing laying hens (REIS, 2022).

Keeping laying hens in cages offers significant benefits in terms of production control, management, and bird health. This results in economic advantages such as reduced labor required, less waste and feed costs. In addition, the use of cages allows the automation of egg feeding and collection, which facilitates handling (GUIMARÃES E DE ALMEIDA, 2021). The conventional system lowers the risks of dirty eggs, which in turn reduces the spreading of microbes and food that is more reliable (MAHMOUD et al., 2022; REIS, 2022).

For the conventional system, a minimum space of 310cm^2 per hen must be provided for three or more birds per cage when birds weigh less than 2.4kg (PAVAN et al., 2005). The feeder must be five cm wide per bird, while drinkers can be of the trough type (2.5cm/bird) or nipples (one for every eight birds). Cages must have floors with adequate inclination to move the eggs to the sideboard. It is essential to use caution when adjusting cage inclination, as an excessive angle can result in damage to the eggs due to breakage, while an insufficient inclination can lead to the accumulation of eggs at the bottom of the cages, increasing the amount of dirty eggs (PAVAN et al, 2005).

However, the conventional cage system has been questioned due to the restriction of movements and expression of birds' behaviors, due to the lack of space and compromised ambience, which would harm the welfare of the laying hens (GROOT and VIZÚ, 2021).

2.2. *Cage-free* system

Global egg consumers demonstrate growing concern about animal welfare, reflected in the preference for eggs from production systems that adopt *cage-free* farming practices for laying hens. They believe that birds need to be loose, scratch, take sand baths, flap their wings, lay eggs in nests, and walk freely to express their natural behavior (PORTELA et al., 2019).

The *cage-free* system is monitored and must comply with animal welfare guidelines and the requirements of certifiers, as well as other systems for raising *free-range* chickens. *Cage-free* creations need, to contain perches, nests, lines for drinkers and feeders inside (DE MOURA et al., 2022).

Compared to the conventional system, the *cage-free* system requires a change in birds' management and, therefore, more labor. In Brazil, there is no specific welfare legislation for the breeding of laying hens in a *cage-free* system (PORTELA et al., 2019).

Brazil has protocols for agricultural practices that meet international standards; among them are the Protocol of Egg Production Practices, prepared by the Brazilian Association of Animal Protein (UBA, 2008) and Technical Circular nº 49 – Production Practices in the Commercial Position (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA, 2006). Certified Humane Animal Care (2018) established the guidelines to produce eggs in a *cage-free* system, to obtain certification for the property.

The *cage-free* system is the most trending alternative, as it is easier to implement, due to the possible adaptation of the broiler breeding, adding nests for egg production (PORTELA et al, 2019). However, there are some research reports on negative impacts on production characteristics, including laying percentage, reduced consumption, cannibalism, mortality, and egg quality, causing financial losses. losses (Elson and Croxall, 2006; Englmaierova et al., 2014; Jones et al., 2015; Karcher et al., 2015; Dong et al., 2017).

In order to mitigate possible challenges in poultry production, it is advisable to adapt the composition of the diet through the inclusion of zootechnical additives, such as probiotics, prebiotics, acidifiers, polyphenols, organic acids, among others. This approach aims to promote preventive health, resulting in the preservation of production efficiency and egg quality.

3. Obtaining Purified Lignin

Lignin is a complex and abundant component found in the cell wall of woody plants (NEUTELINGS, 2011; BES et al., 2019; DORTE, 2019). In recent years, obtaining and purifying lignin has attracted significant interest due to its potential as a valuable renewable resource to produce a wide range of chemical, material, and food products (HUANG et al., 2019; FERNANDES, 2021).

Lignin purification is an essential process to obtain high quality lignin from lignocellulosic feedstocks. There are several methods for purifying lignin, the most common being the Kraft method, the Soda method and the Organosolve method (TORRES et al., 2020; KNAPP, 2020). Each one of these involves specific steps to separate lignin from other biomass components.

The lignin purification process by the Kraft method is widely used in the pulp and paper industry (MARTINS, 2023). In this method, lignin is extracted during the process of cooking wood in an alkaline solution of sodium sulfide and sodium hydroxide. During cooking, lignin dissolves and is separated from cellulose and hemicellulose fibers. After that, the lignin is recovered from the alkaline solution through acid precipitation. This process results in obtaining Kraft lignin, which has high purity, but may also contain some unwanted components, such as residues of chemical products used in the process (MAHMOOD et al., 2018; TORRES et al., 2020; KNAPP, 2020; MARTINS, 2023).

The Soda method, also known as the mild alkaline purification method, is another process used to purify lignin (SANTOS, 2021). In this method, lignin is extracted from biomass using an alkaline sodium hydroxide solution. The dissolved lignin is then recovered by acidification, without the need for harsh chemicals. This process results in a lignin with a lower degree of chemical modification compared to Kraft lignin, maintaining some natural properties of the original lignin (MAHMOOD et al., 2018; TORRES et al., 2020; SANTOS, 2021b).

The Organosolve method is a purification process that uses organic solvents in combination with an acid separating agent (SALVE, 2020). In this method, the biomass is treated with an organic solvent, such as ethanol or acetone, in a pressurized atmosphere and elevated temperature. The solvent dissolves lignin and other soluble components, while cellulose and hemicellulose remain insoluble. After that, the lignin is separated from the solvent through distillation or precipitation, resulting in a purified lignin (MAHMOOD et al., 2018; SALVE, 2020).

Each lignin purification method has specific advantages and disadvantages, depending on the desired application and the desired properties of the lignin. The choice of the most appropriate purification method depends on characteristics of the raw material, the lignin purity requirements, and the intended applications (MAHMOOD et al., 2018; HUANG et al., 2019; SALVE, 2020). There are continuous advances in research for new lignin purification methods, to develop better efficiency and quality of purified lignin, making it a promising alternative for a wide range of industrial applications (NEUTELINGS, 2011; MARTINS, 2023).

Purified lignin has been studied in the animal feed industry as an additive with the potential to improve the quality of animal diets. Its inclusion in feed can bring significant benefits as a feed additive, especially due to its antioxidant properties and the capacity to improve the intestinal health and animal welfare (BAURHOO et al., 2007a; MAHMOOD et al., 2018). Research in this area aims to explore and better understand the potential of purified lignin as a promising option for improving animal health and performance through its use in feed formulation (BEZERRA, 2019; RÖHE et al., 2020; SUN et al., 2022).

4. Polyphenols in the diet of laying hens

Polyphenols are bioactive compounds found in a variety of plant foods such as fruits, vegetables, grains, herbs, and spices (SCICUTELLA et al., 2021; LIMA, 2021). These compounds have been the subject of increasing interest due to their potential health benefits and their antioxidant and anti-inflammatory properties. In the diet of laying hens, the inclusion of polyphenols can have positive effects in several areas, from the health of the birds to the quality of the eggs produced (LIPORI, 2019; ZHOU et al., 2021; ABD EL-HACK et al., 2023).

Studies have shown that the supplementation of the diet of laying hens with polyphenols can improve hens' performance. This includes an increase in laying rate, higher egg weight, increase percentage of production and improved egg quality (WANG et al., 2017; OMER et al., 2019; FAN et al., 2021; LIMA, 2021). Polyphenols have antioxidant properties, which means they can help reduce oxidative stress in bird cells, protecting them against damage caused by free radicals (MARCHIORI et al., 2019; LIU et al., 2020; WANG et al., 2020). Furthermore, these compounds may also have anti-inflammatory effects, aiding the overall health of birds (OHISHI et al., 2016; LIPORI, 2019).

The inclusion of polyphenols in the diet of laying hens can also influence the quality of eggs produced. Studies have shown that polyphenol supplementation can improve yolk color, making it more vibrant and attractive to consumers (LIPORI, 2019; ZHU et al., 2020; FAN et al., 2021). In addition, polyphenols can help strengthen eggshells, reducing the incidence of broken shells and improving structural strength. This is important to ensure eggs' integrity and commercialization (EL-MOTAAL et al., 2008; GOLIOMYTIS et al., 2018; FERNANDES, 2022).

Another benefit of including polyphenols in the diet of laying hens is the transfer of these compounds to the eggs (LIPORI, 2019). The polyphenols present in the food consumed by birds are absorbed and metabolized by their organism, being deposited in the tissues and, consequently, the eggs (WILLIAMSON and CLIFFORD, 2017). Which means that those who consume these eggs can also benefit from the bioactive compounds, gaining the potential positive health effects associated with polyphenols (FERNANDES, 2022; VLAICU and PANAITE, 2022).

4.1. Purified lignin polyphenols

Purified lignin is composed of a variety of polyphenols, which are derived from the polymerization of phenolic units. The main polyphenols found in purified lignin include

(FENGEL and WEGENER, 1983; CONSTANT et al., 2016; BEZERRA, 2019; HUANG et al., 2019):

- Syringyl: It is one of the most common polyphenols in lignin. It has a structure based on syringyl units, which are methoxylated phenolic units.
- Guaiacyl: It is also an important component in purified lignin. Its structure is based on guaiacyl units, which are hydroxylated phenolic units.
- P-Hydroxyphenyl: It is a polyphenol derived from p-hydroxyphenyl units, which are hydroxylated phenolic units.

These polyphenols are present in the complex three-dimensional structure of lignin, which is formed by linking phenolic units through carbon-carbon and carbon-ether bonds (BEZERRA, 2019). The proportion and exact composition of polyphenols may vary depending on the source of lignin and the purification method used (LIPORI, 2019).

It is important to highlight that purified lignin can be obtained from different sources of biomass, such as wood, agricultural residues, and other lignocellulosic materials. Each biomass source may have a slightly different polyphenol composition, which will influence the properties and applications of the resulting purified lignin.

5. Egg quality

Egg quality is a crucial issue for both poultry producers and consumers. Egg quality is influenced by several factors, including bird genetics, age, nutrition, handling conditions, bird health and production environment (DE OLIVEIRA et al., 2019). Egg quality assessment covers different characteristics, such as size, weight, shell color, white and yolk quality, nutrient content, and freshness (RODRIGUES et al., 2019).

Nutrition plays a key role in egg quality, as a balanced and adequate diet is essential to provide the necessary nutrients for the proper development of eggs (GARCIA and GOMES, 2019). The most important nutrients in the diet of laying hens include proteins, amino acids, lipids, vitamins, and minerals (RODRIGUES et al, 2019). Deficiencies or excesses of nutrients can negatively affect egg quality (GARCIA and GOMES, 2019).

The quality of the eggshell is a fudamental aspect. Composed mainly of calcium carbonate and proteins, and it plays a crucial role as a natural protection for the internal contents of the egg (SAMPAIO et al., 2022). In addition, the shell has a physical protection function, contributing to the quality and safety of food.

Furthermore, egg freshness is an essential factor for food quality and safety. Freshness depends on several factors, such as the rearing system, age and nutrition of the hens and storage of the eggs (OLIVEIRA et al., 2020; MORAES, 2021). Adequate refrigeration is necessary to prolong eggs' shelf life, thus reducing the proliferation of microorganisms and quality degradation (DE OLIVEIRA et al., 2019; SAMPAIO et al., 2022).

Egg shelf life is a critical variable in the poultry industry, with a direct impact on egg quality and food safety (DIAS, 2020). Knowledge about what affects the shelf life of eggs is essential to ensure the sale of safe and high-quality products to consumers (SILVA, 2022). Several factors can affect eggs' shelf life, consequently influencing their quality and freshness over time.

Eggs initial quality is an essential factor. High quality eggs, with intact shells and without defects, tend to have a longer shelf life than low quality ones, which may have cracks in the shell, facilitating the entry of microorganisms and accelerating deterioration (DE MEDEIROS et al. al., 2017).

The age of the laying hen also plays an important role. Eggs from older hens tend to be of lower quality, with more fragile shells and more liquid whites, which can reduce shelf life compared to eggs from younger hens (CAMARGO, 2019). The proper handling of eggs, from collection to storage is utmost importance. Eggs must be handled with care, avoiding falls and impact that could damage the shell. In addition, hygiene during the collection process is essential to prevent contamination by dirt or waste (DE OLIVEIRA et al., 2019).

Microbiological control is essential to ensure egg quality and freshness. The presence of microorganisms can lead to deterioration of eggs, and storage at a higher ambient temperature accelerates chemical reactions and the multiplication of microorganisms, hence reducing eggs' shelf life (SILVA, 2022). Control measures must be implemented through the type of system, handling, nutrition, and storage, to prevent external and internal bacterial contamination (BRITO, 2022). Controlling these factors throughout the production process is critical to maximizing shelf life and ensuring the delivery of fresh, high-quality products to consumers.

6. Heat stress in laying hens production

Heat stress is a significant challenge in poultry production, negatively affecting its welfare, health, and performance. During prolonged exposure to high temperatures, chickens face difficulties in regulating their body temperature, which leads to a series of physiological and behavioral changes (CARVALHO, 2019; MARINHAGO, 2020).

During heat stress period, birds can experience a series of negative physiological responses, such as increased body temperature, changes in energy metabolism, immune dysfunction and increased oxidative stress (MAHMOUD et al., 1996; MASHALY et al., 2004). Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant capacity, leading to cell damage (LARA and ROSAGNO, 2013; VANDANA et al., 2021).

The effects of heat stress on production and reproduction in chickens are significant. The reduction in feed intake result in less weight gain, reduced production, and compromised egg quality, consequential in economic losses (CARVALHO, 2019; PEREIRA, 2022). In addition, there is interference in the development and functioning of the reproductive organs, affecting the function of the ovaries and egg production (IRÚN and TECH, 2021; PEREIRA, 2022).

Hens exposed to heat stress exhibit adaptive behavioral responses such as seeking out cooler areas, reduced physical activity, reduced social interaction, and changes in feeding patterns and water consumption (APPLEBY et al., 2004; BHADAURIA et al., 2014).

Another important aspect to be considered is the effect of heat stress on the intestinal microbiota of chickens. Elevated temperature can lead to changes in the composition and balance of the microbiota, which compromises intestinal health and nutrient absorption (LARA and ROSAGNO, 2013; CARVALHO, 2019; VANDANA et al., 2021). This can lead to an increased susceptibility to infection and an overall reduction in hens health.

Nutrition plays a crucial role in mitigating heat stress. The formulation of balanced diets, with adequate levels of nutrients and supplementation of antioxidants, can help reduce the damage caused by heat stress (LOPES, 2019) Antioxidants such as vitamin C, vitamin E and polyphenols have protective properties that help birds to deal with oxidative stress resulting from heat stress (MELLO, 2020; SOUZA, 2020).

The use of dietary polyphenol supplementation may help reduce heat-induced oxidative stress. They act as antioxidants, neutralizing ROS and protecting cells against oxidative damage. In addition, polyphenols have anti-inflammatory properties, which can help lower the inflammatory response caused by heat stress (HALLIWELL, 2008; PROCHÁZKOVÁ et al., 2011; SURAI, 2014; LIPIŃSKI et al., 2017; HU et al., 2019).

Several studies have demonstrated the beneficial effects of dietary polyphenols during heat stress in poultry. For example, the supplementation of plant extracts rich in polyphenols in diets for broiler chickens exposed to heat stress has shown improvements in productive performance, reduction of oxidative stress, modulation of immune response and improvement of intestinal integrity (LIU, HE et al., 2014; OKE et al., 2017; LUO et al., 2018).

In egg production, the inclusion of polyphenols in the diet has also shown positive effects during heat stress. Studies have reported improvements in egg production, eggshell quality, egg lipid profile and yolk oxidative stability (DOSOKY et al., 2021). In addition, polyphenols can also improve the birds' immune response, strengthening their ability to deal with infectious challenges during heat stress (NAWAB et al., 2019).

While the results are encouraging, it is important to note that the effectiveness and effects of polyphenols can vary depending on source, concentration, combination with other dietary ingredients, and duration of supplementation (HU et al., 2019). Therefore, more research is needed to determine the best strategies for using polyphenols in poultry diets during heat stress, considering the specific needs of each species and production phase. This highlights the importance of adding polyphenols to the diet of laying hens.

Thus, the present study aimed to evaluate the effects of including purified lignin in the diet of commercial laying hens in different systems and heat-stressful environments by evaluating productive performance (production, weight and mass of egg, feed intake and feed conversion ratio); internal and external quality of the eggs (lipid peroxidation of the yolk, egg specific gravity, shell thickness, shell strength and percentage of albumen, yolk and shell); morphology of the digestive tract (weight and length); histology of the small intestine and concentration of short-chain fatty acids in the cecal content.

In Chapter 2 contains the study that aimed to evaluate the effects of purified lignin as a feed additive in the diet of laying hens from 31 to 43 weeks, to measure the productive performance, gut morphology, egg quality, and shelf life, entitled: “Effect of supplementation of laying hens from 31 to 43 weeks of age with a diet containing purified lignin as an additive on laying performance, egg quality and shelf life”, which was written by in accordance with the standards of the **Journal of Applied Poultry Research (JAPR)**.

In Chapter 3 contains the study that objective was to verify the production, quality, and composition of eggs from hens supplemented or not with purified lignin in the *cage-free* system, entitled: “Effect of purified lignin on performance, egg quality, intestinal morphology, and histology of laying hens on *cage-free* system”, which was written by in accordance with the standards of the **Journal of Applied Poultry Research (JAPR)**.

In Chapter 4 contains the study that the premise was evaluate the effects of purified lignin and its antioxidant potential in the diet of laying hens in a *cage-free* system, under thermal stress, through productive, physiological and egg quality parameters, entitled: “Effect of purified lignin on performance, egg quality, gut morphology, and histology of laying hens at *cage-free* system under heat stress”, which was written by in accordance with the standards of the **Journal of Applied Poultry Research (JAPR)**.

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