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Effect of Various By-products on the Egg Quality of Laying Hens (*Gallus domesticus*)

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ABSTRACT

This study was aimed to investigate the effects of byproducts on egg quality of white layer hens. A total of 18 hens 28-week old were brought to Animal Toxicology Laboratory of Zoology Department, The Islamia University of Bahawalpur, Pakistan. After habituation, hens were acclimatized with the research laboratory conditions for seven days. A total 18 birds of white laying hens were randomly divided to three groups each group contained six hens. The hens were kept at a photoperiod of 16 h/8 h light/dark cycle, and a room temperature of 25 °C ± 2°C. Feed and water were provided ad libitum and the experiment lasted for five weeks. Birds were offered either a basal diet or the basal diet supplemented with 150, 170 or 190 g/kg of egg shells and tea byproducts. As a result of this study, there were significant differences in egg production and egg weight. The number of egg and egg weight were significantly reduced in byproducts group. There were no significant differences in egg length and egg white weight. However egg width and egg shell thickness were significantly decreased in byproducts group. In summary, egg shell and tea byproducts can be used as an alternative feedstuff in laying hen diets at inclusion levels up to 190 g/kg without any negative impact on performance and egg quality traits. Further studies are needed to determine the level of inclusion of byproducts that does not affect performance.

INTRODUCTION

The world has over 23 billion poultry- about three birds per person on the planet and about five times more than 50 years ago. They are kept and raised in a wide range of production systems, and provide mainly meat, eggs and manure for crop fertilization. Poultry meat and eggs are among the most common animal source food consumed at the global level, through a wide diversity of cultures, traditions and religions, making them key to food security and nutrition. Eggs are considered to be a "miracle food", because they contain approximately 40 proteins, including antihypertensive and bactericidal proteins, 18 different amino acids, including nine essential amino acids, stable amino acid composition, optimal proportion of saturated and unsaturated fatty acids, and no carbohydrates or trans fats (Molnár and Szőllősi 2020). Therefore, eggs have been recognized as a reference protein for humans and they have the same biological value as breast milk (Damaziak et al. 2017).

The global production of table eggs has increased by 24.4% over the past decade, bringing production to 76.7 million tons in 2018, which is expected to increase further because of the high demand for animal-originated protein. This high demand has triggered the need for intensive poultry production, which causes an increased incidence of disease, chronic stress, and compromised production (Olobatoke and Mulugeta 2011). Over the past decades, antibiotics have been regularly included in layer diets to enhance the performance and prevent diseases, with the ultimate production of safe and good quality eggs.

At the global level, three main types of poultry production systems can be considered which are broilers, layers and backyard system. According to Global Livestock Environmental Assessment Model the global production of eggs is around 73 million tons and global production of poultry meat is close to 100 million tons. Based on this modeling approach, backyard systems contribute 8% of global egg production and 2% of global meat. The majority (92%) of poultry meat production comes from specialized broiler systems and layers only contribute to 6% of the total. Backyard systems make significant contribution to eggs and poultry meat production in Eastern Europe, South Asia, Sub-Saharan Africa and, to a lesser extent, East Asia and Latin America and the Caribbean (GLEAM, 2016)**.**

Demand for animal derived food is increasing because of population growth, rising income and urbanization and poultry meat has shown the fastest trend in the last decades. The average annual growth rate for poultry meat over the last 50 years was 5% while it was only 1.5% for beef, 3.1% for pork and 1.7% for small ruminants (Alexandratos and Bruinsma 2012). The biggest poultry meat producers are the United States, with almost 20 million tons a year, followed by China, with 18 million tons, the EU and Brazil with about 13 million tons. Global per capita consumption of eggs increased from 4.55 kg to 8.92 kg between 1961 and 2010, while global per capita consumption of poultry meat increased from 2.88 kg to 14.13 kg (Mottet and Tempio 2017).

The potential feed ingredients for poultry include green algae, whole dates and date pits and other plant materials such as barley, oats, rye and wheat (Pourreza, Samie and Rowghani 2007). Corn, rice tips, soybean meal, fish meal, canola meal, rape seed meal, rice polish and guar meal are main ingredients for poultry feed.The addition of appropriate enzymes to feed improve nutrients digestibility and availability for poultry. However barley could be used without enzyme supplementation at up to 20% of the diet in broiler grower and finisher diets. It has no adverse effects on weight gain, feed conversion, live ability and the yield of broiler grown to 42 days. The use of naked oat-based feed mixture for laying hens could improve the intensity of egg production, increase egg weight, reduce number of non-standard eggs and reduce cost of egg production (Hozlar, Valčuhová and Jančík 2014). Using 20% of wheat screenings in broiler rations had no adverse effect on broiler performance and that it could lead to a decrease in ration price. The hatchery by-products can be converted into a nutritionally-dense meal through proper processing and could be used as source of calcium and protein for broilers and layers (Al-Nasser 2006).

Various enzymes are used as feed additives. Exogenous protease supplementation is gaining in popularity in animal nutrition with beneficial effects on growth performance, nutrient digestibility and endogenous enzyme secretion (Olukosi et al. 2015). Tannase enzyme was recently supplemented to. broiler feed in order to improve feeding value of diets containing field beans (Abdulla et al. 2016). Phytase works by releasing some of the indigestible phosphorus (and other nutrients) found in commonly used feed ingredients and making the nutrients available for productive purposes. Amylase digest more of the starch found in corn thus providing more available energy. Xylanase releases energy from the fibrous portion of grains and grain byproducts.

Phytogenic feed additives are a group of natural growth promoters (NGPs) or non-antibiotic growth promoters used as feed additives, derived from herbs, spices or other plant. Phytogenic feed additives (PFA), are incorporated into poultry feed to enhance productivity through the improvement of nutrients digestibility, absorption and elimination of pathogens residents in the animal gut (Athanasiadou, Githiori and Kyriazakis 2007). Phytogenic products of plant origin are natural, less toxic, residue free and ideal feed additives for animal when compared to synthetic antibiotics or inorganic chemicals. Phytogenic substances have antimicrobial, antifungal antiparasitic antiviral, antitoxigenic and insecticidal properties. Phytogenic feed additives are either available in a solid, dried and ground form or as extracts or essential. Humic substances as natural growth enhancers are used for their antioxidant, antifungal, detoxifying, and antiseptic properties (Rath, Huff and Huff 2006). In broilers, the benefits in body weight gain, feed efficiency and feed utilization as well as increasing in the length of villi of the jejunal mucosa and reduction in depth of crypt due to the inclusion of HA (Humic Acid) have been observed (Ozturk and Coskun 2006)**.**

Despite the high demand on poultry products worldwide, the poultry industry is facing numerous challenges on the global level. Some of these include high feed costs (which had a great impact on production cost and subsequently on eggs and meat prices), biofuel production and availability of corn as a major feed ingredient for poultry, poultry diseases, quality of products, antibiotic use in feed, environmental impacts, strong global competition and consumer perception with regards to food safety and animal welfare (Hafez and Hafez 2013)**.** These factors result in annual economic losses of $128 to $165 million in the poultry industry alone. The total annual economic loss is $1.69 to $2.36 billion in the U.S. livestock industry (Nawab et al. 2018).

Feed cost could be reduced indirectly by proper management practices on the poultry farm such as reducing feed waste. This could be done by using only quantities required for optimum production without overfeeding. Feeding birds to match their requirements and to improve the efficiency of nutrient utilization will reduce the nutrient load in the manure, and hence on the

environment (Penz and Bruno 2011**).** In addition, using pelleted or crumbled feed will help in reducing feed waste and improving nutrient utilization (Jahan, Asaduzzaman and Sarkar 2006). The crumble form of feed is better than mash and pellet for the production of commercial broiler for the age duration 21 to 56 days. The pellet diameters of 1.59 or 2.38 mm can be beneficial during the pre-starter period and can be more useful with 2.5% poultry oil (Cerrate et al. 2008). Although improved broiler performance is an advantage for pellet feeding, some disadvantages seem to be connected to this feeding method with respect to animal health, whereby increased growth rate from pellet feeding may increase mortality due to ascites, especially in male birds (Zohair, Al-Maktari and Amer 2012).

MATERIALS AND METHODS

Study Area

The study was performed for a period of 1month from August 2022 to September 2022 in laboratory of Animal Toxicology in the Zoology Department, the Islamia University of Bahawalpur, Pakistan. Bahawalpur is one of the hottest areas of Pakistan situated at 112m above the sea level. The area has very hot and dry climate in summer, dry and cold in winter. The study was approved in full by the ethical review committee for the use of animals which comes under the administrative control of the office of research, innovation and commercialization of the Islamia University of Bahawalpur, Pakistan.

Birds, Housing, Diet and Management

Total 18 birds of white egg laying hens were obtained from a well-reputed poultry farm from district Lodhran (Province Punjab, Pakistan). The layer hens were then transferred to Toxicolgy laboratory in the Zoology Department, the Islamia University of Bahawalpur, Pakistan. After shifting, hens were given the adjustment period to the research laboratory conditions for seven days. Total 18 birds of white laying hens, 28-week of age, were randomly divided to three groups each group contained six hens. The hens were kept at a photoperiod of 16 h/8 h light/dark cycle, and a room temperature of 25 °C ± 2°C. The experiment lasted for five weeks, and the birds were given access to feed and water *ad libitum* (with the inclusion of dietary treatments) throughout the experimental period. Commercially available laying hen feed was offered as a basal diet. The ingredients and calculated the chemical composition of the diet are listed in Table 1.

Table 1

Diet composition of layer hen

Layer ration	
Ingredients	**% composition**
Maize	35
Rice bran	35
Soya cake	10
Fish meal	15
Limestone	02
Ground dried Legume leaves	35
Salt	05
Mineral premix	01
Total	100

Experimental Design

Experiment was performed during the month of August and September, 2022. After seven days hens were divided into three dietary groups composed of 2 experimental groups and 1 control group. Each group contained six replicates. Experimental group 1 was fed with different byproducts such as green tea and egg shells and experimental group 2 was fed with different seeds such as pomegranate seeds, sunflower seeds and foxtail millet. While control group was offered with simple feed to investigate the effects of these feeds on the egg quality.

Physiological Response

The egg weight, length, width of shell, areas of egg shell, yolk weight, white weight, thickness of shell as a physiological response of layer hens was recorded regularly in all three groups.The increase or decrease of egg size in dietary treated groups with compare to the control group were recorded precisely.

Performance Parameters

The eggs were collected manually at 8 AM each day. The weight and number of eggs laid were recorded daily. The egg weight was determined by a HM-200 electronic scale (A&D Co., Ltd., Tokyo, Japan). The abnormal eggs (broken eggs, shell-less, or soft shells) were excluded when measuring the egg weight. The egg production percentage was calculated as the hen-day egg production (HDEP) and egg mass were calculated by multiplying the average egg weight with HDEP. The percentage of broken eggs was calculated by dividing the number of broken and soft-shell eggs by the total number of eggs laid.

Fresh feed and water were offered daily on an *ad libitum* basis. The feed and water intake were determined weekly by measuring the residues. The feed conversion ratio (FCR) was calculated by dividing the feed intake by the egg mass.

Egg Quality Parameters

The egg quality indices were determined every week by randomly collecting 12 eggs per treatment. The eggshell thickness was measured using a Peacock dial gauge (P-1 Model, Meg Co Ltd., Ozaki, Japan) after removing the shell membrane and it is represented as the average thickness of the upper, middle, and lower end of the shell.

The egg length (L) and width (W) were measured using vernier calipers with the least count of 0.01 mm. The egg shape index (SI) was determined from the egg length and width.

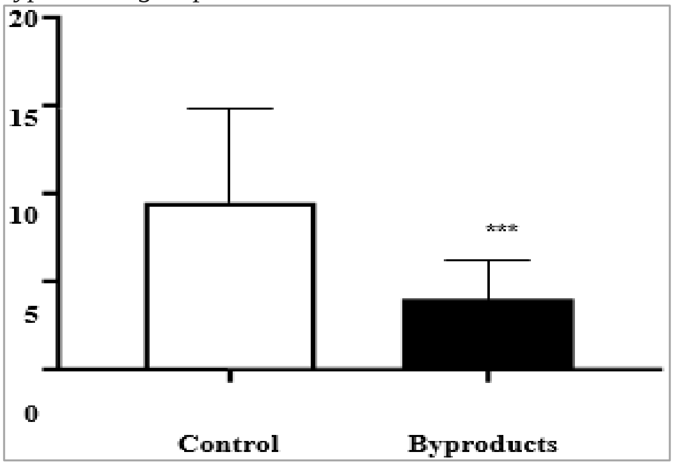
RESULTS

Effect of Byproducts on Egg Numbers

The effect of diet on the egg numbers has shown in the (figure 4.1). A t-test analysis revealed that there was a significant difference in numbers of eggs between the control group and byproducts group (p<0.0155). The numbers of eggs ware significantly reduced in byproducts group.

Figure 1

The effect of byproducts on the egg numbers. The number of eggs were significantly reduced in byproducts group.

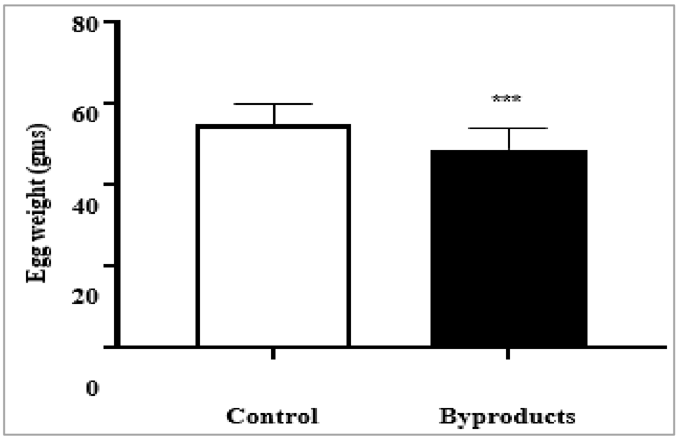


Effect of Byproducts on the Egg Weight

The effect of byproducts is shown in the (figure 4.2). A t-test analysis revealed that there was a significant difference in egg weight between control group and byproducts group (p <0.0.0219). The egg weight was significantly more in control group. However egg weight in byproducts group was significantly reduced.

Figure 2

The effect of byproducts on the egg weight. The egg weight of control group was significantly more while egg weight of byproducts group was significantly reduced.

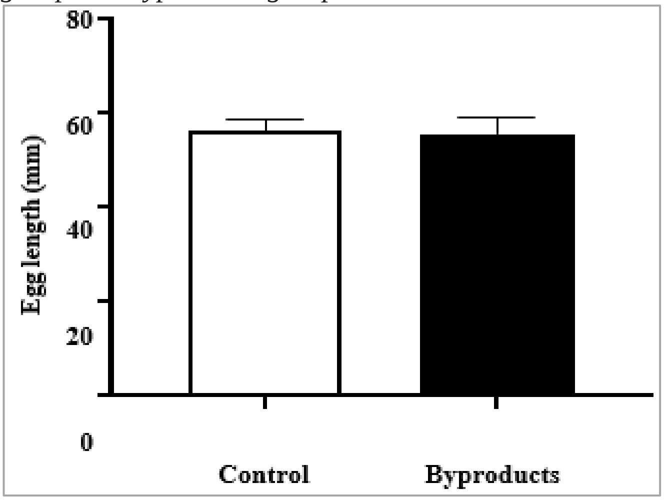


Effect of Byproducts on the Egg Length

The effect of byproducts on the egg length is shown in (figure 4.3). A t-test analysis revealed that there was no significant difference between the control group and byproducts group (p<0.9842). The average length of control group eggs was 56.035mm while average egg length of byproduct group was 56mm as shown in the graph (fig 4.3).

Figure 3

The effect of byproducts on the egg length. There was no significant difference in egg length between control group and byproducts group.

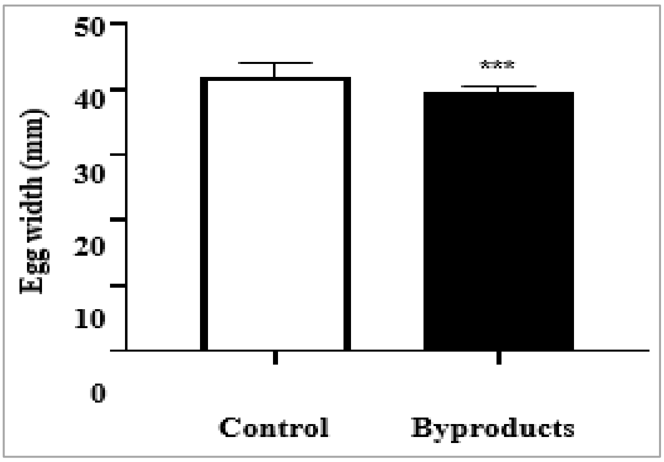


Effect of Byproducts on the Egg Width

The effect of byproducts on the egg width is shown in the (figure 4.4). A t-test analysis revealed that there was a significant difference between control group and byproducts group (p <0.0.4438). The egg width of control group was significantly while egg width of byproduct group was significantly reduced. The average width of control group eggs was 41.42mm while average width of byproduct group eggs was 40.31mm as shown in the (figure 4.4).

Figure 4

The effect of byproducts on the egg width. The width of control group eggs significantly increased. However the egg width of byproducts group was significantly decreased.

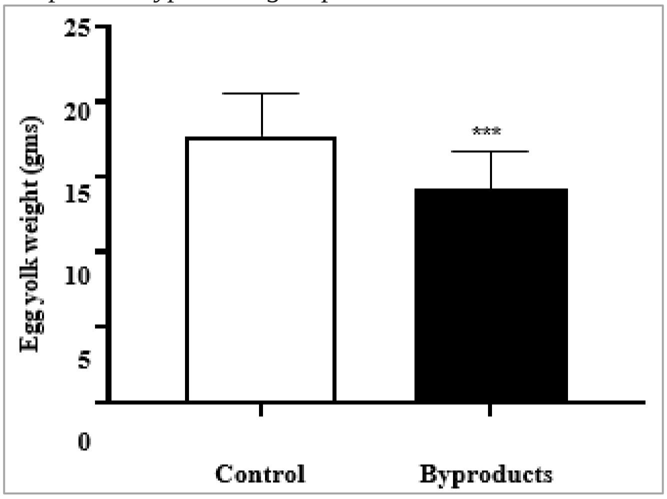


Effect of byproducts on egg yolk weight

The effect of byproducts on the egg yolk weight is shown in the (figure 4.5). A t-test analysis revealed that there was a significant difference in egg yolk weight between control group and byproducts group ($p < 0.0049$). The egg yolk weight of control group significantly increased as compare to byproducts group. The average yolk weight of control was 17.405gms while average yolk weight was 16.3gms of byproduct group as shown in the (figure 4.5).

Figure 5

The effect of byproducts on the egg yolk weight. The yolk weight of control group eggs significantly increased as compare to byproduct group.

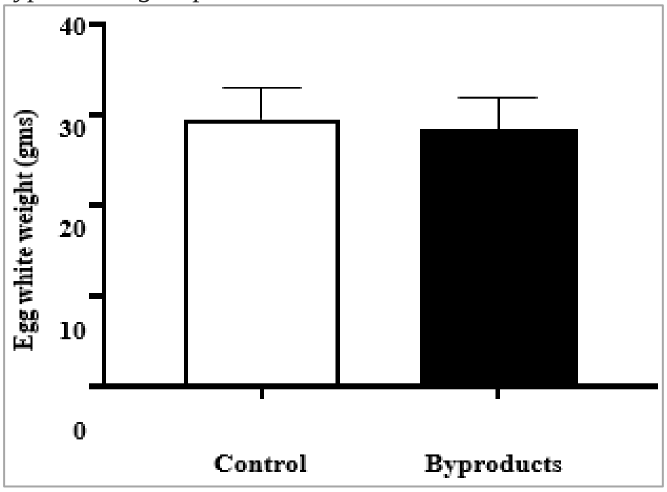


Effect of Byproducts on the Egg White Weight

The effect of byproducts on the egg white weight is shown in the (figure 4.5). A t-test analysis revealed that there was no significant difference between control group and byproducts group ($p < 0.6300$). The average egg white weight of control group was 29.027gms while the average egg white weight of byproducts group was 28.426gms as shown in the (figure 4.6).

Figure 6

The effect of byproducts on the egg white weight. There was no significant difference between control group and byproducts group.

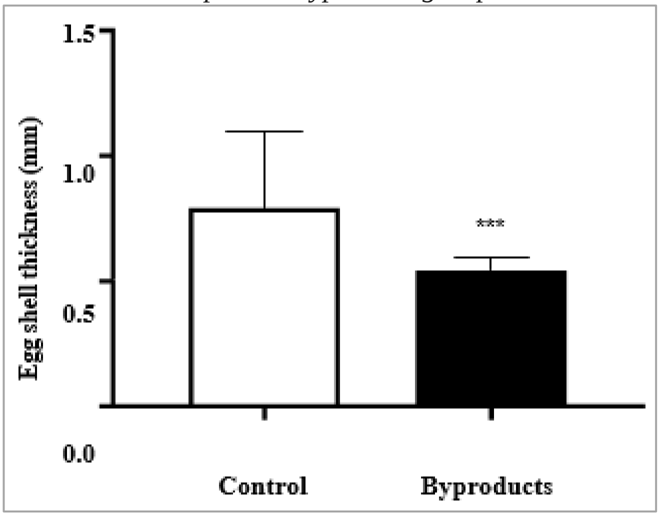


Effect of Byproducts on Egg Shell Thickness

The effect of byproducts on the egg shell thickness is shown in (figure 4.7). A t-test analysis revealed that there was a significant difference between control group and byproducts group ($p < 0.0469$). The egg shell thickness of control group was significantly more as compare to byproducts group. The average egg shell thickness of control was 0.651mm while average egg shell thickness was 0.7mm of byproduct group as shown in the (figure 4.7).

Figure 7

The effect of byproducts on the egg shell thickness. The egg shell thickness of control group eggs significantly increased as compare to byproduct group.



DISCUSSION

In the current study, the birds were provided with egg shells and tea waste had reduced egg production. These results contradict with the results of Abdel-Wareth and Lohakare, who reported that feeding peppermint to laying birds had a positive influence on the conversion of digested feed into eggs, which is crucial for the oviposition process. No effect of feeding egg shell and tea waste byproducts was observed on overall hen-day egg production (Abdel-Wareth and Lohakare 2014). (Shumye et al. 2022) reported that there was nonsignificant effect in the mean number of eggs produced per hen which were supplemented with banana peel. Egg shells contain a high amount of fibers similar to other byproducts such as sugar beet pulp, soy hulls or coffee husks. To date, there is little knowledge on effect of egg shells on laying hen performance and egg quality. Hens are more tolerant to dietary fibers, and diets containing 3.12 to 3.88% of crude fiber mainly from sugar beet pulp linearly increased feed intake and egg production of hens during 25 to 33 week of age (Selim and Hussein 2020). In the present study, hens fed 7.5 to 15% of egg shells in the diet, equivalent to 2.48 or 5.55% of crude fiber, had no negative effects on egg production. Similar results were found in literature that fibers from soy hulls or coffee husks (higher content of pectin) reduced the hen body weight, but did not influence egg product (Sousa et al. 2019).

The outcomes of this research revealed that egg weight has significantly decreased in byproducts group. Diet supplementation with the extract from onion had a positive effect on the mean egg weight, in contrast to the supplementation with the garlic extract, which had no effect on this trait. The supplementation of egg shells and tea byproducts in diet of layer hen has no significant effect on the egg width. These results were similar with the results of Dilawar et al. who reported that supplementation of plants extract byproducts in layer hen diet has no significant difference in egg width, egg height, and eggshell thickness (Dilawar et al. 2021). The findings of this research also showed no significant effect of egg shells and tea byproducts on the egg yolk and egg white weight. Egg quality refers to various standards that define both external and internal quality. The internal quality is focused on the yolk height, yolk color, albumin viscosity, and Haugh unit. In contrast, the external quality refers to the eggshell thickness, egg width, and height and cleanliness (Coutts and Wilson 2007). The eggshell thickness is associated with the proportion of damaged eggs during transport and handling (Anderson et al. 2004). The present study observed a significant difference in eggshell thickness between control group and byproducts group. The shell thickness of control group significantly increased as compare to byproducts group. Previously, Abdel-Wareth and Lohakare reported that peppermint oil increased the eggshell thickness, which was found in the present study (Abdel-Wareth and Lohakare 2014).

The results suggested that tea byproducts supplementation had adverse effect on strength and thickness of eggshell. These findings were consistent with Kojima and Yoshida study, which showed that the addition of tea by-products decreased the eggshell strength (Kojima and Yoshida 2008). It could be related to insufficient nutrition absorption especially the inadequate absorption of calcium, or tea treatment altered the process of anabolism and catabolism during eggshell formation. Current studies proved that exterior nutrition could affect the material metabolism, and then cause some changes of egg quality (Liu et al. 2018). Off course, there were some different results suggesting that tea resource treatment improved the eggshell thickness and egg specific gravity (Wei *et al.*, 2012). Although some inconsistent findings were reported, nutrition indeed could change the metabolism, and then alter egg quality. (Olgun, Cufadar and Yildiz 2009) suggested that reasonable regulating exogenous nutrition may serve as a pathway to improvement in egg quality.

The eggshell thickness of the layers fed diets containing green tea powder was significantly thinner than that of the control group. But, no significant differences were observed in eggshell thickness of groups offered 0.5 to 2.0% green tea and antibiotics diets. This result was similar to (Yang, Jung and Uuganbayar 2003) who reported that eggshell thickness was reduced slightly when layers were fed diets containing 2.0 to 6.0% green tea by-product supplementations. Research has shown that various factors, including the age of the birds, their diet, and genetic background, can influence egg weight (Yakubu, Ogah and Barde 2008). The inclusion of varying percentages of tomato by-products in chick diets led to notable alterations in specific biochemical parameters. This resulted in increases in ash, fat, and carbohydrate levels, while protein content and energy decreased. The extent of these changes was influenced by the proportion of tomato by-products incorporated into the diet (Boulaajine et al. 2024).

A greater egg weight resulting from genetic selection was linked to an increased albumen content, which aligns with findings observed in most breeds examined. Additionally, the yolk content was found to be statistically significant, varying according to breed and line (Hejdysz et al. 2024). Data on external and internal egg quality parameters revealed that certain traits, such as weight, pH, strength, and temperature, were significantly affected by both group and temperature. Moreover, the interaction between these factors also influences egg quality, particularly for attributes like yolk weight, pH, and shell thickness, highlighting the complex relationships at play (Mashaly et al. 2004). Yolk and albumen weights were influenced by egg weight groups, with their values increasing proportionally as egg weight increased (Tebesi, Madibela and Moreki 2012).

The storage period significantly impacted egg weight loss, shell weight, shell thickness, air cell size, and albumen pH (Samli et al. 2006). After an egg is laid, physico-chemical changes (egg aging) cause the shell to lose its protective properties, allowing water and gases to move internally and externally. Water loss is faster in smaller eggs due to their greater surface area relative to volume (Lewko and Gornowicz 2011). The color of yolks in laying hens is mainly determined by the types and amounts of carotenoid pigments found in their feed. This color can be easily modified by adjusting the feed ingredients to align with consumer preferences (Islam et al. 2017). Supplementing laying hen diets with 4% to 6% grape pomace appears to have no negative effects on performance or egg quality and may enhance egg shelf life by lowering MDA levels in the yolk, although further research is needed on its impacts on animal health and digestion (Kara et al. 2016).

Study also demonstrated that laying hens can safely tolerate up to 6% tomato waste meal (TWM) in their diet without negatively impacting egg production or quality. At this level, TWM enhanced the egg yolk color score, lowered yolk cholesterol, and increased the concentration of lycopene in the yolk. However, if TWM inclusion exceeds 6%, egg production may decline, which could also be linked to reduced feed energy. The yolk color score progressively improved as the levels of TWM in the diet increased (Habanabashaka, Sengabo and Oladunjoye 2014). The quality of egg yolks, assessed through the Yolk Index of broken eggs or by evaluating yolk centering and roundness during candling, tends to change at a similar rate as R. R. Haugh units or other indicators of albumen condition. One of the key factors influencing egg quality is how the eggs are handled (Stadelman 2017).

CONCLUSION

In the current study, the effect of byproducts (egg shell and tea byproduct) on the egg quality of layer hen was investigated. It is concluded that egg shells and tea byproducts as a diet supplementation to layer hen has significantly decreased egg weight and number of eggs. However, egg production has increased significantly in control group. The egg weight, egg yolk weight and egg width significantly increased in control group as compare to byproducts group. There was no significant difference in egg length and egg width between control group and byproducts group. The egg yolk weight and egg shell thickness were significantly affected by egg shell and tea byproducts. These byproducts (egg shell, tea byproducts) could be used as alternative feed for layer hen. These byproducts have no adverse effect on the layer hens. However, more research is required to fully understand the effect of egg shells and tea byproducts on the egg quality of layer hens.

Author's Contribution

Adeeba Naseer: Conceptualization, Investigation; Formal Analysis, Methodology, Visualization. Writing - original draft: Sumaira Raziq: Data Curation, Investigation, Muhammad Muneer, Muhammad Usman, Maqsood Ahmad, Muhammad Asad Ullah, Ayesha Khadim, Hafsa Mushtaq, Hafiz Muhammad Mubashar Ali and Tarkan Şahin: Writing, Review & Editing.

Availability of Data and Material

All the data is available and can be obtained on reasonable request from the corresponding author

Consent to Participate

All the authors equally participated in this research.

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