Journal of Health and Rehabilitation Research 2791-156X JHRR

For contributions to JHRR, contact at email: editor@jhrlmc.com

*Original Article*

# Investigating the Impact of Marigold Supplementation on Egg Yolk Color Intensity: A Study on Dietary Additives

**Sajjad Hussain[1], Esha Gulfreen[1], Sobia Abid[1], Shawana Khalil[2], Muhammad Rizwan[3], Nimra Batool[4], Abdul Aziz[5], Yousra[1], Hafiz Muhammad Usman Abid[6], Nasir Mahmood[2]***

[1]Department of Zoology, The Islamia University of Bahawalpur, Pakistan.
[2]Department of Zoology, Faculty of Sciences, University of Central Punjab, Pakistan.
[3]Multan College of Veterinary Sciences, Multan University of Science and Technology Multan, Pakistan.
[4]Government College University Faisalabad, Pakistan.
[5]Department of Veterinary Clinical Sciences, University of Poonch, Rawalakot, Pakistan.
[6]Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.
*Corresponding Author: Nasir Mahmood; Email: mahmoodnasir445@gmail.com*

***Conflict of Interest: None.***

*Hussain S., et al. (2024). 4(1): **DOI:** https://doi.org/10.61919/jhrr.v4i1.712*

## ABSTRACT

Background: The inclusion of marigold flower powder (MFP) in the diets of layer hens, a rich source of xanthophylls, presents a natural method to enhance egg quality. This study examines the effects of MFP on egg characteristics including yolk color, overall weight, and the weights of individual egg components.

Objective: To assess the impact of dietary supplementation with 4% MFP on egg yolk color, egg weight, albumin content, yolk weight, shell weight, and hen body weight in layer hens.

Methods: Ten 27-week-old layer hens were divided into two groups: a control group (T0) and a treatment group (T1), with each group containing five replicates. The T1 group's diet was supplemented with 4% MFP. The study was conducted over a 60-day period, with measurements taken in a completely randomized design. Egg quality metrics (weight, yolk weight, albumin weight, and shell weight), yolk color (evaluated using the Roche Yolk Colour Fan, or RYCF), and hen weight were measured and analyzed on the sixty-first day.

Results: The study found no significant difference ($P>0.05$) in internal egg qualities (egg yolk weight and egg albumin weight) or hen weight between the T0 and T1 groups. However, significant improvements were observed in external egg qualities; egg weight and shell weight showed a significant increase ($P<0.05$) on the 45th day of the trial. Moreover, egg yolk color in the T1 group was significantly enhanced compared to T0, achieving a RYCF score of 12.66.

Conclusion: Dietary supplementation with marigold flower powder significantly enhances the external qualities of eggs, notably egg weight and shell weight, as well as the color intensity of the egg yolk. These findings suggest that incorporating MFP into layer hens' diets is an effective natural strategy to improve egg quality. Future research should consider an economic analysis of incorporating MFP for pigment enhancement in layer hens' diets.

Keywords: Carotenoids, Egg yolk color, Marigold, Nutritional Enhancement, Ocular Health, Pigments, Xanthophyll.

## INTRODUCTION

Egg yolk composition is significantly influenced by the diet of layer hens, which makes them an interesting topic for investigation in functional food research (1). In this context, enhanced eggs supplemented with folic acid, vitamins B12, D, E, and n-3 fatty acids have drawn interest (2). Interestingly, carotenoids, especially lutein and zeaxanthin, which are known to have the ability to prevent cataracts and age-related macular degeneration, are found naturally in eggs (3). Egg yolk colour is greatly affected by carotenoids, which in turn affects consumer perception and reflects dietary habits (4).

With albumin (63%), eggshell (9.5%), and yolk (27%), eggs are primarily composed of protein (75%) and lipids (12%) (5). They are also a vital source of vitamins, minerals, and carbohydrates (6). Consumer preferences for taste, nutritional value, and freshness are just a few of the qualities of egg quality that are frequently correlated with yolk colour. The vivid colour of egg yolks is highly favored worldwide, influencing consumer choices and product innovation (7).

Journal of Health and Rehabilitation Research 2791-156X JHRR

Although hens cannot make pigments on their own, they do accumulate them in their yolks from food, mainly from carotenoids (8). The colour of the yolk is greatly influenced by xanthophyll sources from plants like marigold, such as lutein and zeaxanthin. Supplements containing xanthophylls are frequently used in poultry farming to improve the colour of the yolk (9). From the standpoints of producers, consumers, and processors, yolk coloration is extremely significant and significantly influences egg quality parameters such as shelf life, visual appeal, and sensory qualities.

To achieve the desired yolk coloration, producers often add feed additives that are enriched with red and yellow carotenoids. Marigold is widely used for egg pigmentation because of its high pigment concentration and abundant xanthophyll content. African marigold, which is highly valued for its lutein content, is particularly important for improving yolk coloration (10). Loved for its antioxidant qualities, marigold extract is often added to chicken feeds to increase the colour and carotenoid content of the eggs (11, 12). The purpose of the study is to assess the effects of feeding marigold to layer hens on various aspects of egg quality, such as albumin weight, yolk coloration, and hen performance (13).

This study's exploration of marigold supplementation in layer hen diets and its subsequent impact on egg yolk pigmentation extends significant benefits to human health sciences. By enhancing egg yolk color through natural carotenoids such as lutein and zeaxanthin, derived from marigold, the research directly contributes to the nutritional enrichment of eggs. These carotenoids are pivotal in human diet for their proven roles in preventing cataracts and age-related macular degeneration, offering a natural defense against these prevalent visual impairments. Consequently, the study not only addresses avian dietary improvements but also emphasizes a feasible, dietary approach to bolster human eye health and antioxidant intake, highlighting the interconnectedness of poultry nutrition and human health benefits.

## MATERIAL AND METHODS

The investigation into the effect of marigold supplementation on egg yolk pigmentation in Gallus gallus domesticus was meticulously designed and executed at the University of Central Punjab, Bahawalpur. This locale, characterized by its extreme climatic variations, spans 24,830 km^2 and is situated at an elevation of 152 meters above sea level, providing a unique environment for the study.

Twelve egg-laying hens of the species Gallus gallus domesticus, aged 27 weeks, were procured from the Government Poultry Farm in Bahawalpur for the purpose of this study. These hens were then allocated into two distinct groups, labeled T0 and T1, with each group comprising one rooster and five hens. The T0 group was designated as the control, while the T1 group received the experimental intervention.

The housing for these birds was constructed by the University of Central Punjab, featuring cages that measured six feet in length and four feet in width. These enclosures were equipped with ample water provisions and designed to ensure natural lighting and ventilation, thus simulating a conducive living environment for the hens.

The experimental intervention centered around the inclusion of marigold flower powder (MFP) in the diet of the T1 group. The marigold flowers, sourced from the local market in Bahawalpur, were dried and ground into a powder before being stored in plastic bags. Prior to the initiation of the experimental diet, all hens were fed a basal diet, outlined in Table No. 1, to ensure uniformity in nutritional intake. This basal diet was administered twice daily, and the average daily feed consumption was recorded at 120g per hen.

Table 1

| Ingredient | Diet % |
|---|---|
| Total Carotenoid, mg/g | 25.1 |
| Total Xanthophyll's, mg/g | 32.15 |
| Lutein, mg/g | 22.56 |
| Zeaxanthin, mg/g | 1.65 |

*Basal diet of layer hen*

| Ingredients | g/kg | composition | g/kg |
|---|---|---|---|
| Wheat, ground (105g CP/kg) | 366 | Dry matter | 891 |
| Maize, ground (83 g CP/kg) | 50 | Crude protein | 175 |
| Soybean meal, extracted (450 g CP/kg) | 90 | Crude fat | 60 |
| Barley, ground (120g CP/kg) | 200 | Crude fiber | 33 |
| Soybean oil | 7 | Calcium | 37 |
| Limestone | 82 | Total phosphorus | 5.9 |

The experimental diet commenced with the inclusion of 4% MFP, which was meticulously measured using an electronic weighing machine to ensure precision. The marigold powder was mixed with flour and water before being added to the hens' diet, thus forming the experimental diet that was fed twice daily.

To address potential health concerns and ensure the well-being of the hens throughout the study, a comprehensive disease control protocol was implemented. This included the administration of vidaylin and Calcibex for vitamin and mineral deficiencies, amoxil and brufen for temperature regulation, and the ND lasota vaccine for immunization against the novel castle disease, which was reapplied every fourteen days.

Egg collection was performed daily, with eggs from each group being marked and stored separately to facilitate the evaluation of egg quantity and quality. Following a fifteen-day period of dietary supplementation, the eggs were counted, and their weight was measured using an electronic weighing machine. Additionally, three eggs from each group were selected for detailed analysis of quality parameters, including shell weight, yolk weight, albumin weight, and yolk color. The shell and internal components were weighed separately, and the yolk color was assessed using the Roche Yolk Colour Fan (RYCF), a standardized tool ranging from 1 (very light yellow) to 15 (very dark yellow). Observations noted an initial yolk color rating between 1 and 2, which increased to 4.66 after the first fifteen days, indicating a significant impact of the dietary supplementation on yolk pigmentation.

Further qualitative assessment was conducted through a panel test involving custard and a full boiled egg derived from both T0 and T1 groups. Eight professors from various departments were selected as panelists to ensure an unbiased evaluation, employing a scorecard that ranged from excellent (90 to 100) to poor (60 to less than 70), based on the yolk color intensity and uniformity.

The weight of the hens was another parameter monitored at the onset and every fifteen days thereafter. Initial measurements indicated an average weight of 1.38 kg, with subsequent readings showing no significant fluctuations ($p>0.05$), thereby suggesting that the dietary supplementation did not adversely affect the hens' body weight.

Statistical analysis was conducted using the Statistics 8.1 software, version 2021. A two-way factorial design analysis of variance (ANOVA) was utilized to determine the significance of the observed effects, with a p-value of less than 0.05 indicating statistical significance.

This comprehensive methodology, characterized by its systematic approach and adherence to rigorous scientific standards, facilitated a thorough investigation into the effects of marigold supplementation on egg yolk pigmentation, thus contributing valuable insights to the field of zoological nutrition.

## RESULTS

### Analysis of yolk color

The colour of the egg yolk was significantly affected by the tested marigold treatment ($P<0.05$), according to the analysis of variance table. Table displays the highly significant ($P<0.05$) yolk colour among the days and treatments. When 4% marigold flower powder was added to the diet, the yolk colour increased. Roche, a yolk colour enthusiast, a standardized tool, was used to assess the yolk colour. The yolk colour is rated from 1 (very light) to 15 (very dark orange). The hen's diet significantly impacted yolk colour. The marigold flower was the primary colour pigment score in the traditional diet. When comparing T1 to T0, the yolk colour was significantly different ($P<0.05$). The experiment's maximum value for egg yolk colour was noted after 60 days. On Roche fan colour, the maximum score for yolk colour was 12.66.

| Impact of different treatment at different day's interval on yolk color of hen | | | | | | Mean values of Yolk color (RYCF) in layer hens with 4% MFP for 60 days in captivity (mean ± SEM). | | |
|---|---|---|---|---|---|---|---|---|
| SOV | DF | SS | MS | F-value | P-Value | Days | T0 | T1 |
| Days | 4 | 118.467 | 29.91 | 32.91 | 0.000 | 0 | $1.66^{d}$ | 2.33 |
| Treatment | 1 | 128.133 | 142.133 | 142.37 | 0.0000 | 15 | $3.00^{d}$ | 4.66 |
| Days*Treatment | 4 | 72.86 | 18.217 | 20.24 | 0.0000 | 30 | $2.00^{d}$ | $5.33^{c}$ |
| Error | 20 | 18.217 | 0.900 | | | 45 | 3.00 | $8.00^{b}$ |
| Total | 29 | 337.467 | | | | 60 | $3.00^{d}$ | $12.66^{a}$ |
| SOV = Source of variation; DF = Degree of freedom; SS = Sum of Squares; MS = Mean Squares; p < 0.05 Highly-significant <0.01 | | | | | | P<0.05 T0= Control T1= 4%MFP | | |

Journal of Health and Rehabilitation Research JHRR

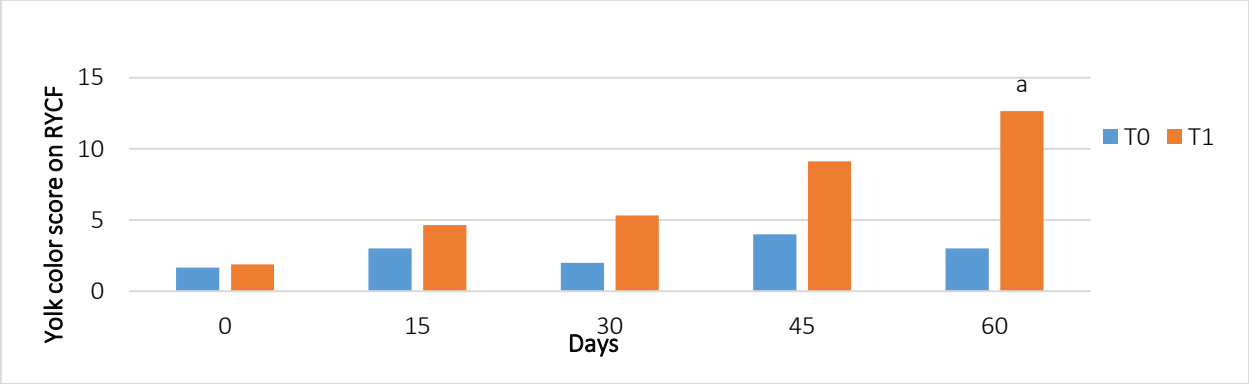


*Figure 1:* Score of Yolk color on RYCF (mean ± SEM)

## Egg weight and shell weight

The days and treatments indicated in Table are significantly impacted by egg weight, according to an analysis of variance. The findings showed that a 4% MFP increase in the hen's egg weight was achieved. Egg weight was significantly (P<0.05) impacted by 4% MFP. The experiment's maximum egg weight was noted after 60 days. The maximum egg weight, measured with an electronic balance, was 51.30 g. The days and treatments indicated in the table had been substantially impacted by shell weight, according to an analysis of variance. Shell weight significantly increased (P<0.05) after 4% MFP was added to their diet. The experiment's 45 and 60-day shell weight values were recorded at their maximum values. At the 45-day mark of the experiment, the shell weight value was 6.51. Table displays the mean table illustrating the impact of 4%MFP on shell weight.

| Impact of different treatment at different day's interval on Egg weight | | | | | | Mean values of Egg weight in layer hens with 4% MFP for 60 days in captivity (mean ± SEM). | | |
|---|---|---|---|---|---|---|---|---|
| SOV | DF | SS | MS | F-value | P-Value | Days | T0 | T1 |
| Days | 4 | 150.530 | 37..537 | 44.90 | 0.0000 | 0 | $43.60^{d}$±0.90 | $43.27^{d}$±0.79 |
| Treatment | 1 | 30.361 | 30.361 | 36.22 | 0.0000 | 15 | $43.62^{d}$±0.549 | $44.46^{cd}$±0.58 |
| Days*Treatment | 4 | 18.151 | 4.5376 | 5.41 | 0.004 | 30 | $45.60^{c}$±0.55 | $48.60^{cd}$±0.13 |
| Error | 20 | 16.765 | 0.8382 | | | 45 | $43.52^{d}$±0.52 | $45.12^{b}$±1.00 |
| Total | 29 | 215.809 | | | | 60 | $48.30^{b}$±0.005 | $51.30^{a}$±1.15 |
| SOV = Source of variation; DF = Degree of freedom; SS = Sum of Squares; MS = Mean Squares; p < 0.05 Highly-significant P<0.01 | | | | | | P<0.05 T0 = Control T1 = 4% MFP | | |

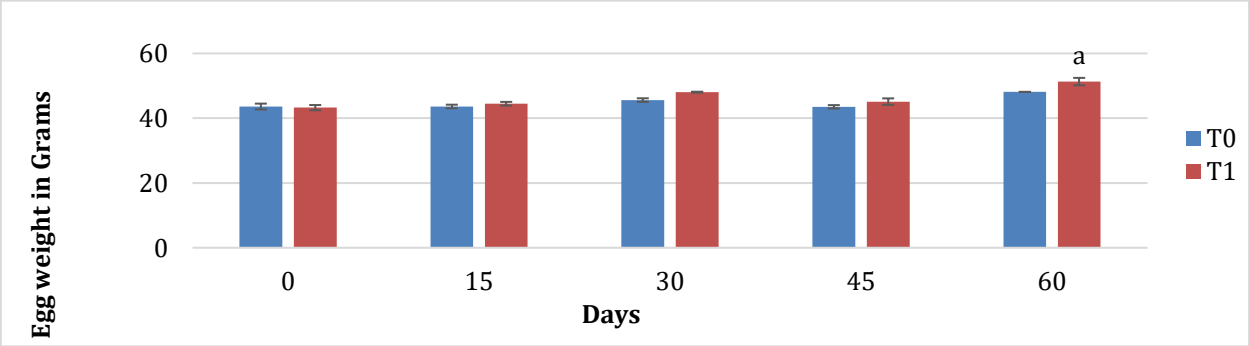


*Figure 2:* Egg weight in grams (mean ± SEM).

| *Impact of different treatment at different day's interval on Shell weight* | | | | | | Mean values of shell weight in layer hens with 4% MFP for 60 days in captivity (mean ± SEM) | | |
|---|---|---|---|---|---|---|---|---|
| SOV | DF | SS | MS | F-value | P-Value | Days | T0 | T1 |
| Days | 4 | 8.35219 | 2.08805 | 9.08 | 0.0003 | 0 | $4.65^{cd}$±0.45 | $4.54^{cd}$±0.62 |
| Treatment | 1 | 2.74867 | 2.74867 | 11.96 | 0.0026 | 15 | $5.39^{bc}$±0.72 | $5.41^{bc}$±0.65 |
| Days*Treatment | 4 | 0.78451 | 0.78451 | 3.41 | 0.0290 | 30 | $4.46^{d}$±0.56 | $6.11^{ab}$±0.12 |
| Error | 20 | 0.22986 | 0.22986 | | | 45 | $5.96^{ab}$±0.31 | $6.51^{a}$±0.40 |
| Total | 29 | | | | | 60 | $5.10^{cd}$±0.1 | $6.25^{ab}$±0.29 |
| SOV = Source of variation; DF = Degree of freedom; SS = Sum of Squares; MS = Mean Squares; VR = Variance Ratio; P < 0.05 | | | | | | P<0.05 T0 = Control T1 = 4% MFP | | |

Journal of Health and Rehabilitation Research JHRR

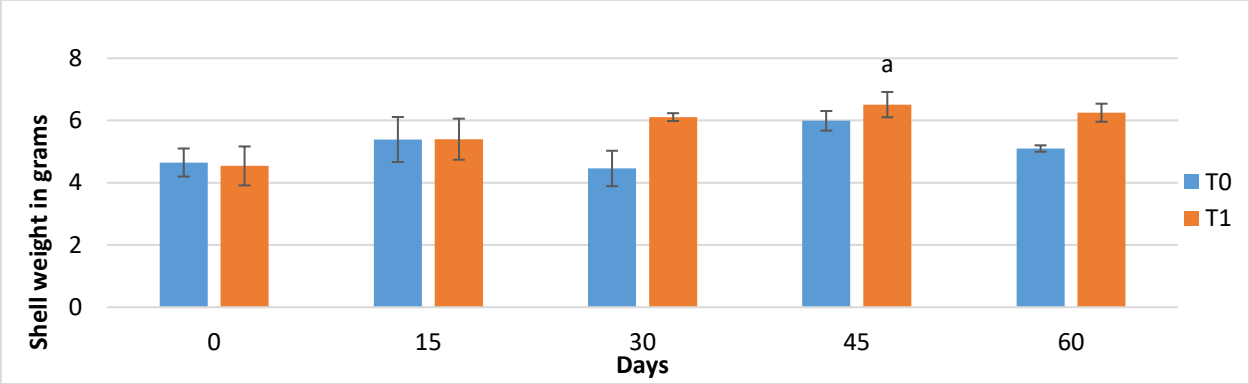


*Figure 3:* Shell weight in grams (mean ± SEM).

### Yolk weight and Albumin weight

According to the analysis of variance, neither days nor treatment significantly affected yolk weight, as shown in Table. Throughout the experiment, the yolk weight was measured every fifteen days. Days and treatment were likewise shown to produce non-significant results. Table displays the mean table for the impact of MFP on albumin weight. Figure 5 displays the albumin weight graph.

| *Impact of different treatment at different day's interval on Yolk weight* | | | | | | *Mean values of yolk weight in layer hens with 4% MFP for 60 days in captivity (mean ± SEM).* | | |
|---|---|---|---|---|---|---|---|---|
| SOV | DF | SS | MS | F-value | P-Value | Days | T0 | T1 |
| Days | 4 | 30.7225 | 7.68061 | 15.14 | 0.0000 | 0 | $13.11^{cd}$±0.29 | $12.9^{d}$±0.20 |
| Treatment | 1 | 0.0998 | 0.09976 | 0.20 | 0.6622 | 15 | $14.12^{bc}$±0.82 | $15.27^{ab}$±1.10 |
| Days*Treatment | 4 | 3.4083 | 0.85206 | 1.68 | 0.1940 | 30 | $12.94^{cd}$±0.02 | $13.92^{cd}$±0.65 |
| Error | 20 | 10.1441 | 0.50720 | | | 45 | $15.13^{a}$±0.45 | $15.80^{ab}$±0.49 |
| Total | 29 | 44.3745 | | | | 60 | $15.80^{a}$±0.41 | $15.06^{a}$±0.89 |
| SOV = Source of variation; DF = Degree of freedom; SS = Sum of Squares; MS = Mean Squares; $p < 0.005$ | | | | | | P>0.05 T0 = control T1 = 4% MFP | | |

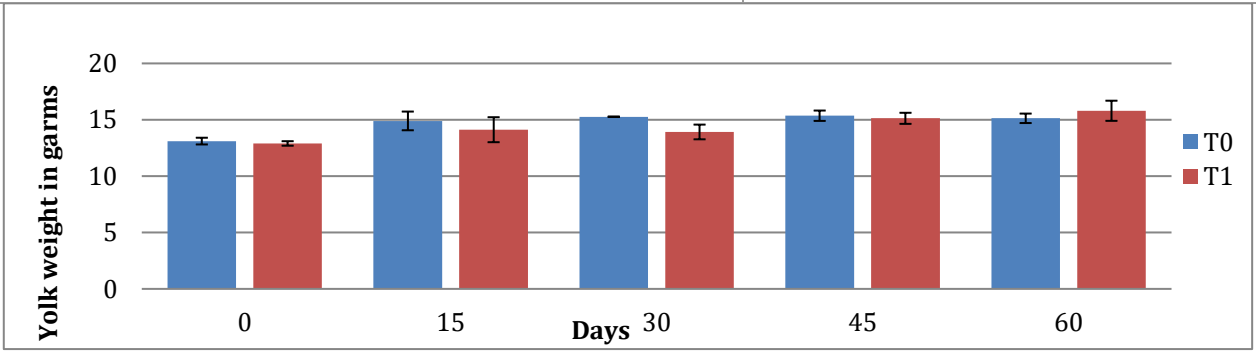


*Figure 4:* Yolk weight in grams (mean ± SEM).

| *Impact of different treatment at different day's interval on Albumin weight* | | | | | | *Mean values of Albumin weight in layer hens with 4% MFP for 60 days in captivity (mean ± SEM).* | | |
|---|---|---|---|---|---|---|---|---|
| SOV | DF | SS | MS | F-value | P-Value | Days | T0 | T1 |
| Days | 4 | 6.9448 | 1.73621 | 1.44 | 0.2575 | 0 | $26.29^{ab}$±1.26 | $25.87^{ab}$±0.62 |
| Treatment | 1 | 0.0760 | 0.07600 | 0.06 | 0.06 | 15 | $27.44^{a}$±0.74 | $26.28^{ab}$±1.94 |
| Days*Treatment | 4 | 4.5924 | 1.14811 | 0.95 | 0.95 | 30 | $26.41^{ab}$±1.02 | $27.13^{ab}$±1.00 |
| Error | 20 | 24.1139 | 1.20570 | | | 45 | $25.28^{b}$±0.73 | $26.28^{ab}$±0.95 |
| Total | 29 | | | | | 60 | $26.76^{ab}$±1.20 | $27.19^{a}$±0.90 |
| SOV = Source of variation; DF = Degree of freedom; SS = Sum of Squares; MS = Mean Squares; $p < 0.05$ | | | | | | *P*<0.05 T0 = control T1 = 4% MFP | | |

Journal of Health and Rehabilitation Research 2791-156X JHRR

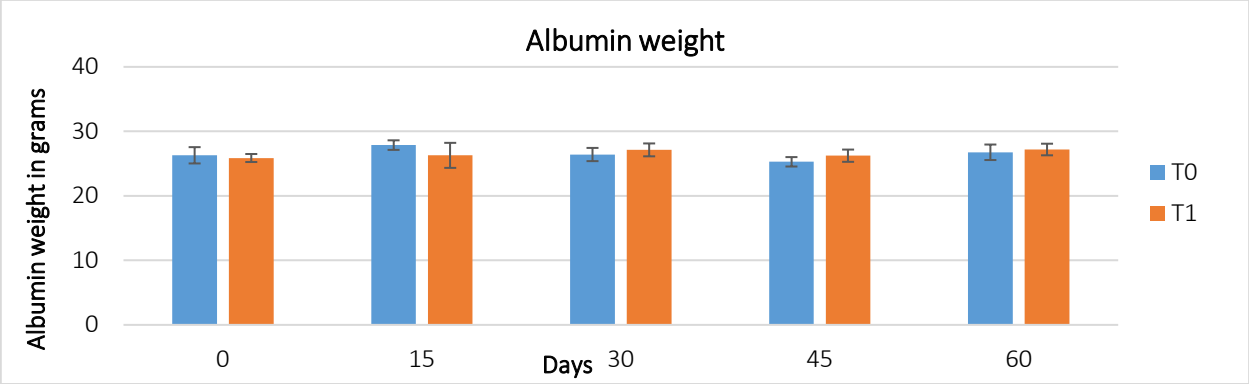


*Figure 5:* Albumin weight in grams (mean ± SEM).

### Hen weight

Hen weight did not significantly affect the days and treatments indicated in table, according to analysis of variance. The outcome, treatment, and number of days were all non-significant (P>0.05). Moreover, the outcome shows that the hens' final body weight was unaffected by the 4% MF addition to their diet. The weight of the hen was not significant (P>0.05). Throughout the trial, the weight of the hen was noted every 15 days. The weight of the hen was reset to zero (1.38 kg). The mean table for the impact of MF on the body weight of hens demonstrated that the outcome with treatment and the outcome with days were both non-significant.

| *Impact of different treatment at different day's interval on Hen weight* | | | | | | *Mean values of Hen weight in layer hens with 4% MFP for 60 days in captivity (mean ± SEM).* | | |
|---|---|---|---|---|---|---|---|---|
| SOV | DF | SS | MS | F-value | P-Value | Days | T0 | T1 |
| Days | 4 | 0.01698 | 0.00424 | 0.36 | 0.8329 | 0 | $1.38^{b}$±0.004 | $1.58^{a}$±0.32 |
| Treatment | 1 | 0.007701 | 0.07701 | 6.56 | 0.0186 | 15 | $1.37^{b}$±0.01 | $1.45^{ab}$±0.04 |
| Days*Treatment | 4 | 0.01982 | 0.00495 | 0.42 | 0.7908 | 30 | $1.41^{ab}$±0.04 | $1.51^{ab}$±0.06 |
| Error | 20 | 0.23473 | 0.01174 | | | 45 | $1.41^{ab}$±0.01 | $1.48^{ab}$±0.01 |
| Total | 29 | 0.34855 | | | | 60 | $1.43^{ab}$±0.02 | $1.49^{ab}$±0.02 |
| SOV = Source of variation; DF = Degree of freedom; SS = Sum of Squares; MS = Mean Squares; $P < 0.05$ | | | | | | p>0.05 T0 = control T1 = 4% MFP | | |

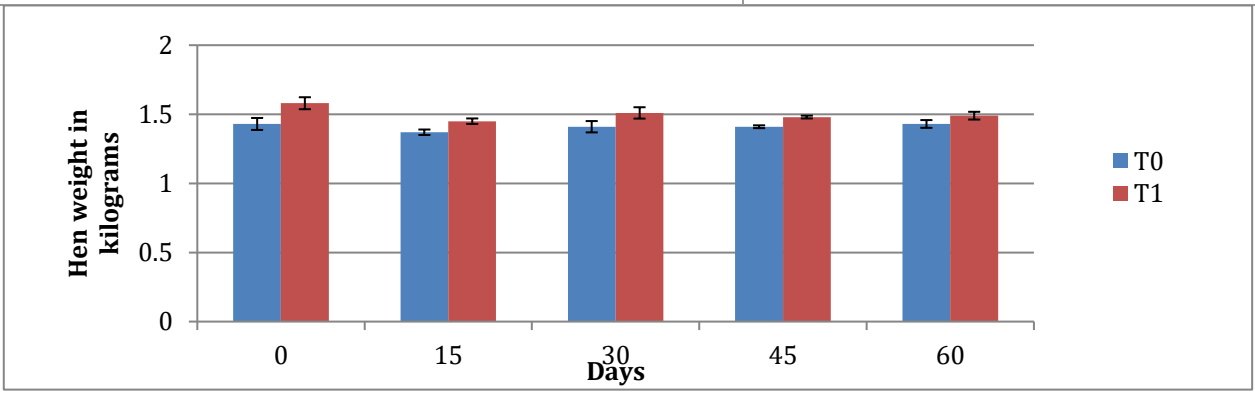


*Figure 6:* Hen weight in kilograms (mean ± SEM).

## DISCUSSION

The purpose of this study was to examine the use of 4% MFP (marigold flower powder) in the diet of layer hens as an egg yolk colourant. It is well known that hen nutrition primarily affects yolk colour. Roche yolk colour fan is used to determine yolk colour (14). In this investigation, the treated group's egg yolk underwent a significant (p<0.05) alteration in comparison to the control group. The treated group had the brightest yolk colour, scoring 12.66, while the control group scored 3.66. A highly significant difference in yolk colour was observed after 60 days of the experiment. This outcome is consistent with a previous study that found marigold to increase yolk colour (15). Marigold had a major impact on the colour of the yolk. Egg internal quality, or the colour of the yolk, increased when marigolds, which are high in lutein, were consumed (15). 4% MFP resulted in an increase in yolk colour. They found

Journal of Health and Rehabilitation Research 2791-156X JHRR

that the layer hens with an egg yolk colour score of 12.00 had a higher score than the paprika group. Feeding laying with 4% marigold meal resulted in an 11.00 score for yolk colour; however, the current study's score was 12.00 with 4% MFP. In the meantime, after adding 1.2% marigold to the diet, the hen's yolk colour improved within 10 to 15 days. Egg yolk colour was enhanced by the marigold supplement's high xanthophyll and lutein content. When xanthophyll-containing diets are added, yolk colour increases. Carotene affects the colour of the yolk in eggs. Egg pigmentation includes 4200 mg/kg of carotenoids. While marigold is a significant source of xanthophyll, zeaxanthin and lutein are the primary xanthophylls responsible for the colour of egg yolks (16). Egg weight increased significantly ($P<0.05$) with 4% MFP in our study. At day 60 of the trial, the highest egg weight was 51.30g ($P<0.05$). Previous research indicated that marigold increased egg weight. In their investigation, the control group's values were lower when marigold extract supplementation was used. E1 (marigold extract) was used to determine the egg with the highest weight. Supplementing with lutein increased the weight of the eggs. Moreover, the variation was not statistically significant. The marigold contains lutein; however, lutein did not have an impact on the weight of the eggs. Egg weight was not significantly affected by marigold. In our study, the treated egg's shell weight significantly increased when compared to the control. The result for shell weight was significant ($P<0.05$). In the previous study, the addition of marigold did not significantly affect shell weight; however, in our study, the egg weight increased, leading to an increase in shell weight. Because marigold contains lutein, there was an increase in egg weight. The weight of the shell increased along with the weight of the egg.

The performance of the layer hen, including yolk colour, egg weight, and egg production, was impacted by the addition of 4%MFP (17). Other characteristics of the eggs, such as albumin weight, hen body weight, and yolk weight, were not significant ($P>0.05$). Yolk weight and albumin weight were not significant ($P>0.05$) in the current investigation. According to earlier research, the results of 4% MFP are non-significant ($P>0.05$). The weight of the hen's yolk, body weight, and albumin were not significantly affected by marigold at different dosages of 10, 20, 30, or 40 mg per kg of diet. The albumin, yolk, and body weights were unaffected by 40 mg of marigold. According to D Lantzouraki et al., the use of 40 mg/kg of marigold had no discernible effect on the weight of the yolk, albumin, hen, or shell(4). The quality of the eggs was affected by marigold flower extract. The body weight, albumin weight, and yolk weight of the hen were not significantly ($P>0.05$) affected by 4% MFP in our study. The weight of the yolk and shell are affected by either 4% marigold or 4% paprika. These results are consistent with earlier research, which indicated that the quality of the eggs remained unchanged. Numerous researchers found that supplementation did not significantly alter production performance.

This study's insights into marigold supplementation and egg yolk pigmentation have direct implications for human health sciences, especially in the context of dietary enhancements(18). The increased yolk pigmentation, attributed to the higher concentrations of lutein and zeaxanthin from marigold, underscores a potential strategy for naturally fortifying eggs with essential nutrients known to combat ocular diseases, such as age-related macular degeneration and cataracts(19,20). Consequently, the enrichment of eggs through marigold supplementation could offer a practical, dietary means of increasing the intake of these crucial carotenoids, promoting eye health and antioxidant protection in human populations, thereby highlighting the significance of agricultural practices in enhancing human nutritional outcomes.

## CONCLUSION

The weight of the egg yolk, egg albumin, and hen body did not significantly change when marigold was added to the diet of the layer of hens. When layer hens are fed 4% marigold, they can lay eggs with a colour score of 12.66 on the sixtyth day of the experiment. Despite the fact that the marigold's xanthophyll produced the colour of the egg yolk, the marigold's lutein contributed to the increase in egg weight. The egg weight shell also increased significantly ($P<0.05$) as a result of the increase. In conclusion, an economic analysis should be conducted regarding the use of marigold, a natural pigment, in the diet of layer hens. This study's findings on marigold supplementation in hen diets suggest a natural way to enhance dietary carotenoids for humans, potentially improving ocular health and antioxidant intake.

## REFERENCES

1. Saleh AA, Gawish E, Mahmoud SF, Amber K, Awad W, Alzawqari MH, et al. Effect of natural and chemical colorant supplementation on performance, egg-quality characteristics, yolk fatty-acid profile, and blood constituents in laying hens. Sustainability. 2021;13(8):4503.
2. Aleem A, Khan M, Abid U, Ishfaq MF, Rouf A, Jamshaid T. Microbial analysis of selected brands of whitening creams. Saudi J Med Pharm Sci. 2020;6:178-82.
3. Mrowicka M, Mrowicki J, Kucharska E, Majsterek I. Lutein and zeaxanthin and their roles in age-related macular degeneration—neurodegenerative disease. Nutrients. 2022;14(4):827.

Journal of Health and Rehabilitation Research 2791-156X JHRR

4. Lantzouraki D. Study of Bioactive Constituents of Hen Egg Yolks After Receiving Enriched Feeds: Εθνικό και Καποδιστριακό Πανεπιστήμιο Αθηνών (ΕΚΠΑ). Σχολή Θετικών Επιστημών ...; 2020.
5. Réhault-Godbert S, Guyot N, Nys Y. The golden egg: nutritional value, bioactivities, and emerging benefits for human health. Nutrients. 2019;11(3):684.
6. Ahmad M, Khan M, Asif R, Sial N, Abid U, Shamim T, et al. Expression characteristics and significant diagnostic and prognostic values of ANLN in human cancers. International Journal of General Medicine. 2022:1957-72.
7. Berkhoff J, Alvarado-Gilis C, Keim JP, Alcalde JA, Vargas-Bello-Pérez E, Gandarillas M. Consumer preferences and sensory characteristics of eggs from family farms. Poultry science. 2020;99(11):6239-46.
8. Bibi R, Hassan A, Ullah A, Shahid MJ, Ullah U, Adeel M, et al. The Impact of Promix Probiotics on the Growth and Survival of Pangas (Pangasius Pangasius) In the Biofloc System at 40% CP Feed. Central Asian Journal of Medical and Natural Science. 2023;4(6):739-61.
9. Usman Abid HM, Hanif M, Mahmood K, Abid S, Khan M, Khurshid U, et al. Exploring the Potent Combination of Quercetin–Boronic Acid, Epalrestat, and Urea Containing Nanoethosomal Keratolytic Gel for the Treatment of Diabetic Neuropathic Pain: In Vitro and In Vivo Studies. Molecular Pharmaceutics. 2023;20(7):3623-31.
10. Irum Z, Aleem A, Ramzan N, Haq IU, Abid U, Murad S. Food Effects Assessment on Oral Pharmacokinetics of Rosuvastatin in Wild Mice. 2019.
11. Azeem M, Hanif M, Mahmood K, Ameer N, Ramzan N, Abid U, et al. Enhanced antibacterial and antioxidant properties of chitosan-quercetin complex containing polycaprolactone microspheres for the treatment of gastroenteritis: An in-vitro and in-vivo analysis. Materials Today Communications. 2022;31:103780.
12. Hanif M, Ameer N, Mahmood MK, Shehzad A, Azeem M, Rana HL, et al. Improved anti-inflammatory effect of curcumin by designing self-emulsifying drug delivery system. Drug Development and Industrial Pharmacy. 2021;47(9):1432-8.
13. Akhlaq M, Abid HMU, Sajid M, Haq IU, Akhtar MH, Zubair G, et al. Antioxidant, Immunomodulatory and Fungicidal Potential of Different Extracts of Raphanus Raphanistrum L. Var. Caudatus. Journal of Health and Rehabilitation Research. 2024;4(1):646-51.
14. Beardsworth P, Hernandez J. Yolk colour–an important egg quality attribute. International Poultry Production. 2004;12(5):17-8.
15. Lokaewmanee K, Yamauchi K-e, Komori T, Saito K. Effects on egg yolk colour of paprika or paprika combined with marigold flower extracts. Italian Journal of Animal Science. 2010;9(4):e67.
16. Karadas F, Grammenidis E, Surai P, Acamovic T, Sparks N. Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. British poultry science. 2006;47(5):561-6.
17. Wang Z, Kong L, Zhu L, Hu X, Su P, Song Z. The mixed application of organic and inorganic selenium shows better effects on incubation and progeny parameters. Poultry Science. 2021;100(2):1132-41.
18. Sünder A, Wilkens M, Boehm V, Liebert F. Egg yolk colour in organic production as affected by feeding–Consequences for farmers and consumers. Food Chemistry. 2022 Jul 15;382:131854.
19. Mim YZ, Sultana F, Dey B, Ray BC, Nishat NJ. Effects of Marigold Petal and Leaves on Yolk Pigmentation in Laying Hens. Journal of the Bangladesh Agricultural University. 2021 Sep 30;19(3):325-31.
20. Kljak K, Carović-Stanko K, Kos I, Janječić Z, Kiš G, Duvnjak M, Safner T, Bedeković D. Plant carotenoids as pigment sources in laying hen diets: Effect on yolk color, carotenoid content, oxidative stability and sensory properties of eggs. Foods. 2021 Mar 29;10(4):721.